PATENT
Atty. Docket No.: 8576.0068

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

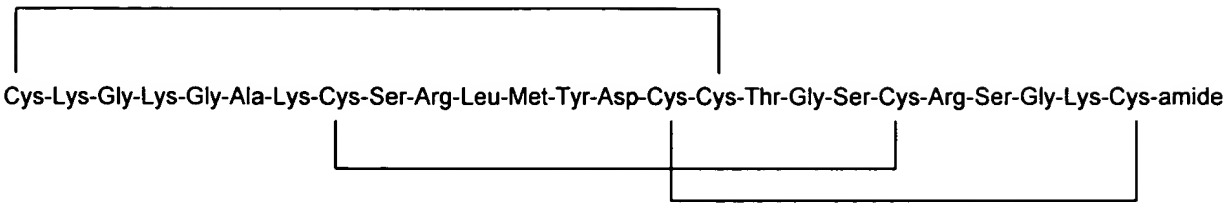
In re U.S. Patent No. 5,795,864)
)
Issued: August 18, 1998)
)
To: Gary Arthur Amstutz, Stephen Scott Bowersox,)
Kishorchandra Gohil, Peter Isadore Adriaenssens,)
Ramasharma Kristipati)
)
Assignee: ELAN Pharmaceuticals, Inc.)
)
For: STABLE OMEGA CONOPETIDE FORMULATIONS)

ATTN: MAIL STOP PATENT EXT.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPLICATION FOR EXTENSION OF PATENT
TERM UNDER 35 U.S.C. § 156

Applicant, ELAN Pharmaceuticals, Inc., represents that it is the Assignee of the entire interest in and to United States Patent No. 5,795,864 granted to Gary Arthur Amstutz, Stephen Scott Bowersox, Kishorchandra Gohil, Peter Isadore Adriaenssens and Ramasharma Kristipati on the 18th day of August, 1998, for Stable Omega Conopetide Formulations by virtue of an assignment from the inventors to Neurex Corporation, recorded in the U.S. Patent and Trademark Office at Reel 7575, Frame 0234 on June 27, 1995, and from Neurex Corporation to ELAN Pharmaceuticals, Inc. recorded at Reel 9689, Frame 0776 on January 4, 1999. By the Power of Attorney enclosed herein (Attachment A), Applicant appoints several individual attorneys, including Charles E. Van Horn, as attorneys for ELAN Pharmaceuticals, Inc. with regard



PRIALT® is formulated as a sterile, preservative-free, isotonic solution that contains ziconotide acetate with L-methionine and sodium chloride as excipients.

(2) The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act Section 505.

(3) The approved product PRIALT® received permission for commercial marketing or use under Section 505 of the Federal Food, Drug and Cosmetic Act on December 28, 2004. A copy of the approval letter for NDA 21-060 is attached (Attachment B).

(4) The active ingredient in PRIALT® is ziconotide which, on information and belief, has not been approved for commercial marketing or use under Section 505 of the Federal Food, Drug and Cosmetic Act prior to the approval of NDA 21-060 by the Food and Drug Administration on December 28, 2004. A copy of the package insert describing the approved product is attached (Attachment C).

(5) This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the permitted 60-day period pursuant to 37 C.F.R. § 1.720(f), said period will expire on February 25, 2005.

(6) The complete identification of the patent for which a term extension is being sought is as follows:

Inventors: Gary Arthur Amstutz, Stephen Scott Bowersox, Kishorchandra Gohil, Peter Isadore Adriaenssens, Ramasharma Kristipati

Patent No.: 5,795,864

Filing Date: June 27, 1995

Issue Date: August 18, 1998

Expiration Date: June 27, 2015

(7) A true copy of the patent is attached (Attachment D).

(8) No reexamination certificate or certificate of correction has been issued on this patent. A copy of a record of maintenance fee payments under 35 U.S.C. § 41(b) is attached (Attachment E).

(9) U.S. Patent No. 5,795,864 claims a formulation comprising the active ingredient ziconotide (an omega conopeptide) in PRIALT®. The applicable patent claims are claims 1, 2 and 4 that are directed to a formulation that contains the active ingredient. The following description demonstrates the manner in which at least one claim reads on the approved product.

Claim 1 reads as follows: A stable omega conopeptide formulation comprising an omega conopeptide and an anti-oxidant composition capable of preventing methionine oxidation.

Ziconotide, the active ingredient in PRIALT®, is an omega conopeptide. Ziconotide is described, for example, as SEQ ID NO:1 (MVIIA/SNX-111) at col. 3, line 17 of the '864 patent. PRIALT® contains L-methionine as an anti-oxidant.

(10) The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

Investigational New Drug Application (IND 45718) for PRIALT® was received by the FDA on July 5, 1994 and became effective on August 4, 1994.

New Drug Application for PRIALT® (NDA 21-060) was submitted on December 28, 1999.

New Drug Application for PRIALT® was approved on December 28, 2004.

(11) A brief description of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to PRIALT® and the dates applicable to these significant activities are set forth in a chronology of events in Attachment F.

(12)(i) Applicant is of the opinion that U.S. Patent No. 5,795,864 is eligible for extension of the patent term under 35 U.S.C. § 156 because it satisfies all requirements for such extension as follows:

(a) 35 U.S.C. § 156(a) - U.S. Patent No. 5,795,864 claims a formulation containing the active ingredient (an omega conopeptide) in PRIALT®.

(b) 35 U.S.C. § 156(a)(1) - U.S. Patent No. 5,795,864 has not expired before submission of this application.

(c) 35 U.S.C. § 156(a)(2) - The term of U.S. Patent No. 5,795,864 has never been extended under 35 U.S.C. § 156(e)(1).

(d) 35 U.S.C. § 156(a)(3) - The application for patent term extension is submitted by the owner of record of the patent in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office.

(e) 35 U.S.C. § 156(a)(4) - The product PRIALT® has been subjected to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. § 156(a)(5)(A) - The commercial marketing or use of the product PRIALT® after the regulatory review period is the first permitted commercial marketing or use under the provision of the Federal Food, Drug and Cosmetic Act (i.e., Section 505) under which such regulatory review period occurred.

(g) 35 U.S.C. § 156(c)(4) - No other patent has been extended for the same regulatory review period for the product PRIALT®.

(12)(ii) Applicant respectfully submits that the length of the extension of patent term for U.S. Patent No. 5,795,864 is 3.36 years (1228 days) pursuant to 35 U.S.C. § 156(c). The length of the extension was determined pursuant to 37 C.F.R. § 1.775 as follows:

(a) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) began on August 4, 1994 and ended December 28, 2004, which is a total of 3801 days, which is the sum of (1) and (2) below:

(1) The period of review under 35 U.S.C. § 156(g)(1)(B)(i), the "Testing Period," began on August 4, 1994 and ended on December 28, 1999, which is 1973 days; and

(2) The period of review under 35 U.S.C. § 156(g)(1)(B)(ii), the "Approval Period," began on December 28, 1999, and ended on December 28, 2004, which is a total of 1828 days.

(b) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in subparagraph 12(ii)(a) above (3801) less:

(1) The number of days in the regulatory review period which were on or before the date on which the patent issued (August 18, 1998) which is 1475 days; and

(2) The number of days during which applicant did not act with due diligence, which is zero (0) days; and

(3) One-half the number of days determined in subparagraph (12)(ii)(a)(1) above after the patent issued (one-half of 498) which is 249 days;

(c) The number of days as determined in subparagraph (12)(ii)(b) (2077 days) when added to the original term of the patent (June 27, 2015) would result in the date of March 4, 2021.

(d) Fourteen (14) years when added to the date of the NDA approval (December 28, 2004) would result in the date of December 28, 2018;

(e) The earlier date as determined in subparagraphs (12)(ii)(c) and (12)(ii)(d) is December 28, 2018;

(f) Since U.S. Patent No. 5,795,864 issued after September 24, 1984, the period of extension may not exceed five years from the original expiration date of June 27, 2015. Five years when added to the original expiration date of the patent would result in the date of June 27, 2020.

(g) The earlier date as determined by subparagraphs (12)(ii)(e) and (12)(ii)(f) is December 28, 2018.

(13) Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) The prescribed fee for receiving and acting upon this application is attached as a check in the amount of $1,120.00. The Director is authorized to charge any additional fees required by this application to Deposit Account No. 06-0916.

to this application for extension of the term of U.S. Patent No. 5,795,864 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

Notice Regarding Multiple Applications

Applicant has filed another application for term extension (U.S. Patent No. 5,364,842) based on the regulatory review period for the product PRIALT®. Applicant will make an election of only one patent in accordance with 37 C.F.R. § 1.785(b) upon receipt of a notice of final determination in these applications from the Patent and Trademark Office.

Information Required Under 37 C.F.R. § 1.740

Applicant hereby submits this application for extension of the patent term under 35 U.S.C. § 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. § 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application will be presented in a format which follows the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations.

(1) The approved product PRIALT® contains ziconotide, a synthetic equivalent of a naturally occurring conopeptide found in the piscivorous marine snail, *Conus magus*. Ziconotide is a 25 amino acid, polybasic peptide containing three disulfide bridges with a molecular weight of 2639 daltons and a molecular formula of $C_{102}H_{172}N_{36}O_{32}S_7$. The amino acid sequence and disulfide bridging pattern are given below:

(15) All correspondence and inquiries may be directed to the undersigned, whose address, telephone number and fax number are as follows:

Charles E. Van Horn
Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
901 New York Avenue, N.W.
Washington, D.C. 20001-4413
Phone: 202-408-4072
Fax: 202-408-4400

(16) Enclosed is a certification that the application for extension of patent term under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and two (2) copies thereof (Attachment G).

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Charles E. Van Horn
Charles E. Van Horn
Reg. No. 40,266

Date: February 22, 2005

Attachments:
Power of Attorney (Attachment A)
Approval Letter (Attachment B)
Package Insert for PRIALT® (Attachment C)
U.S. Patent No. 5,795,864 (Attachment D)
Maintenance Fees Paid (Attachment E)
Chronology of Regulatory Review Period (Attachment F)
Certification of Copies of Application Papers (Attachment G)

PATENT
Attorney Docket No. 8576.0068

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In re U.S. Patent No. 5,795,864)
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Issued: August 18, 1998)
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To: Gary Arthur Amstutz, Stephen Scott Bowersox,)
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Assignee: ELAN Pharmaceuticals, Inc.)
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For: STABLE OMEGA CONOPETIDE FORMULATIONS)

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Commissioner for Patents
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Alexandria, VA 22313-1450

Attachment A

Sir:

POWER OF ATTORNEY BY ASSIGNEE

The undersigned, a representative authorized to sign on behalf of the assignee owning all of the interest in this patent, verifies that ELAN Pharmaceuticals, Inc. is the assignee of the entire right, title, and interest in U.S. Patent No. 5,795,864 (the '864 patent) by virtue of an assignment from the inventors to Neurex Corporation, recorded in the U.S. Patent and Trademark Office at Reel 7575, Frame 0234 on June 27, 1995, and from Neurex Corporation to ELAN Pharmaceuticals, Inc. recorded at Reel 9689, Frame 0776 on January 4, 1999. To the best of the undersigned's knowledge and belief, title to the '864 patent is in the assignee.

The undersigned hereby grants power of attorney to

Charles E. Van Horn, Reg. No. 40,266

James B. Monroe, Reg. No. 33,971

Leslie Boley, Reg. No. 41,490

Nina Ashton, Reg. No. 37,273

Mark Hoch, Reg. No. 35,195

Carl Battle, Reg. No. 30,731

Richard Hake, Reg. No. 37,343

both jointly and separately as attorneys with full power of substitution and revocation to prosecute the application for patent term extension of the '864 patent and to transact all business in the Patent and Trademark Office connected therewith.

Please send all future correspondence concerning this application for patent term extension to Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P. at the following address:

Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.
901 New York Avenue, N.W.
Washington, D.C. 20001-4413

Dated: Feb. 18, 2005

By: _____

Name: Carl W. Battle

Title: Sr. Vice President

Assignee: ELAN PHARMACEUTICALS, INC.

Attachment B

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 21-060

Elan Pharmaceuticals
7475 Lusk Boulevard
San Diego, CA 92121

Attention: Mark Brunswick, PhD
Director, Regulatory Affairs

Dear Dr. Brunswick:

Please refer to your new drug application (NDA) dated December 28, 1999, received December 28, 1999, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Prialt (ziconotide intrathecal infusion), 25 mcg/mL in 20 mL fill vials, and 100 mcg/mL in 1, 2, and 5 mL fill vials.

We acknowledge receipt of your presubmission dated October 29, 1999, and your submissions dated January 12, March 10, 22 (2), and 31, April 6, 7, 12, 14, 17, 20, 24, 27, and 28, May 1, 11, 19, 22 (2), and 26, June 23, July 13, 19, and 24, August 7, September 15, 20 (2), and 29, October 13 (2), November 29, and December 28, 2000, and January 26, February 9, 20, and 28, March 13, 20, 23, and 29, April 9, 20, 26, and 27 (2), May 24, and June 1, 13, 19, 21, 25, and 26, July 11 (3) and 17, August 3 and 16, September 17, October 31, November 2, 20, and 29, and December 21, 2001, January 25, March 8, October 7 and 8, and December 3, 2002, January 27 and 31, February 11 and 20, April 3 and 28, May 2, July 25, August 12, and September 8 and 23, 2003, and January 6 and 16, June 25, August 16, October 20 (2) and 26(2), November 1 and 22, and December 2, 6, 8, 9, 14 (2), 20 (2), and 27, 2004.

Your submission of June 25, 2004, constituted a complete response to our July 25, 2001, action letter.

This new drug application provides for the use of Prialt (ziconotide intrathecal) for the management of severe chronic pain in patients for whom intrathecal (IT) therapy is warranted and who are intolerant of or refractory to other treatment, such as systemic analgesics, adjunctive therapies, or IT morphine.

We have completed our review of this application, as amended and it is approved effective on the date of this letter, for use as recommended in the agreed-upon labeling text.

The final printed labeling (FPL) must be identical to the text for the package insert, immediate container and carton labels submitted December 27, 2004. Marketing the product(s) with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

Please submit an electronic version of the FPL according to the guidances for industry titled *Providing Regulatory Submissions in Electronic Format – NDA* and *Providing Regulatory Submissions in Electronic Format-Content of Labeling*. Alternatively, except for the content of labeling, which must be submitted electronically in PDF format, you may submit 20 paper copies of the FPL as soon as it is available but no more than 30 days after it is printed. Individually mount 15 of the copies on heavy-weight paper or similar material. For administrative purposes, designate this submission "**FPL for approved NDA 21-060.**" Approval of this submission by FDA is not required before the labeling is used.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are deferring submission of your pediatric studies for ages 0-16 years until December 28, 2009.

Your deferred pediatric studies required under section 2 of the Pediatric Research Equity Act (PREA) are considered required postmarketing study commitments. The status of this postmarketing study shall be reported annually according to 21 CFR 314.81. This commitment is listed below.

1. Deferred pediatric study under PREA for the management of severe chronic pain in patients for whom intrathecal (IT) therapy is warranted and who are intolerant of or refractory to other treatment, such as systemic analgesics, adjunctive therapies, or IT morphine in pediatric patients ages 0-16 years.

   Final Report Submission: December 28, 2009

Submit final study reports to this NDA. For administrative purposes, all submissions related to this/these pediatric postmarketing study commitment(s) must be clearly designated "**Required Pediatric Study Commitments**".

In addition, submit three copies of the introductory promotional materials that you propose to use for this product. Submit all proposed materials in draft or mock-up form, not final print. Send one copy to this division and two copies of both the promotional materials and the package insert directly to:

Division of Drug Marketing, Advertising,
and Communications, HFD-42
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

Please submit one market package of the drug product when it is available.

We have not completed validation of the regulatory methods. However, we expect your continued cooperation to resolve any problems that may be identified.

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

If you have any questions, call Sara Stradley, Regulatory Project Manager at (301) 827-7430.

Sincerely,

{See appended electronic signature page}

Robert J. Meyer, MD
Director
Office of Drug Evaluation II
Center for Drug Evaluation and Research
Food and Drug Administration

Enclosure

Elan Pharmaceuticals, Inc.

PRIALT (ziconotide **intrathecal infusion**)

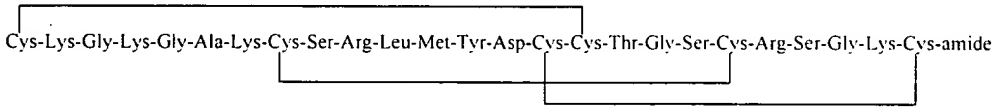
For use only in the Medtronic SynchroMed® EL, SynchroMed® II Infusion System and Simms Deltec Cadd Micro® External Microinfusion Device and Catheter

WARNING:

Severe psychiatric symptoms and neurological impairment may occur during treatment with PRIALT. Patients with a pre-existing history of psychosis should not be treated with PRIALT. All patients should be monitored frequently for evidence of cognitive impairment, hallucinations, or changes in mood or consciousness. PRIALT therapy can be interrupted or discontinued abruptly without evidence of withdrawal effects in the event of serious neurological or psychiatric signs or symptoms.

DESCRIPTION

PRIALT® contains ziconotide, a synthetic equivalent of a naturally occurring conopeptide found in the piscivorous marine snail, *Conus magus.* Ziconotide is a 25 amino acid, polybasic peptide containing three disulfide bridges with a molecular weight of 2639 daltons and a molecular formula of $C_{102}H_{172}N_{36}O_{32}S_7$. The amino acid sequence and disulfide bridging pattern are given below:



Ziconotide is a hydrophilic molecule that is freely soluble in water and is practically insoluble in methyl t-butyl ether.

PRIALT is formulated as a sterile, preservative-free, isotonic solution for intrathecal (IT) administration using an appropriate microinfusion device (See Dosage and Administration). Each 1, 2, or 5 mL vial of PRIALT (100 mcg/mL) respectively contains 100, 200, or 500 mcg of ziconotide acetate, and the 20 mL vial of PRIALT (25 mcg/mL) contains 500 mcg of ziconotide acetate, with

L-methionine and sodium chloride as excipients at pH 4.0–5.0. Each vial is intended for single use only, either undiluted or after dilution to the appropriate concentration with 0.9% Sodium Chloride Injection, USP (preservative free).

CLINICAL PHARMACOLOGY

Pharmacodynamics

Mechanism of Action

Ziconotide binds to N-type calcium channels located on the primary nociceptive (A-δ and C) afferent nerves in the superficial layers (Rexed laminae I and II) of the dorsal horn in the spinal cord. Although the mechanism of action of ziconotide has not been established in humans, results in animals suggest that its binding blocks N-type calcium channels, which leads to a blockade of excitatory neurotransmitter release in the primary afferent nerve terminals and antinociception.

Interaction with opioids

Ziconotide does not bind to opioid receptors and its pharmacological effects are not blocked by opioid antagonists. In animal models, IT ziconotide potentiated opioid-induced reduction in gastro-intestinal (GI) motility, but did not potentiate morphine-induced respiratory depression. In rats receiving IT ziconotide, additive analgesic effects were observed with concurrent administration of morphine, baclofen, or clonidine. Concurrent administration of IT ziconotide and morphine did not prevent the development of morphine tolerance in rats.

PHARMACOKINETICS

The cerebrospinal fluid (CSF) pharmacokinetics (PK) of ziconotide have been studied after one-hour IT infusions of 1–10 mcg of PRIALT to patients with chronic pain. The plasma PK following intravenous (IV) infusion (0.3-10

mcg/kg/day) have also been studied. Both IT and IV data are shown below (Table 1).

Table 1: PRIALT PK Parameters
(Mean ± SD)

Route	Fluid	N	CL (mL/min)	Vd (mL)	$T1/2_{elim}$ (hr)
IT	CSF	23	0.38 ± 0.56	155 ± 263	4.6 ± 0.9
IV	Plasma	21	270 ± 44	30460 ± 6366	1.3 ± 0.3

Following one-hour IT administration of 1 - 10 mcg of PRIALT, both total exposure (AUC; range: 83.6 – 608 ng·h/mL) and peak exposure (Cmax; range: 16.4 – 132 ng/mL) values in the CSF were variable and dose-dependent, but appeared approximately dose-proportional. During 5 or 6 days of continuous IT infusions of PRIALT at infusion rates ranging from 0.1–7.0 mcg/hr in patients with chronic pain, plasma ziconotide levels could not be quantified in 56% of patients using an assay with a lower limit of detection of approximately 0.04 ng/mL. Predictably, patients requiring higher IT infusion dose rates were more likely to have quantifiable ziconotide levels in plasma. Plasma ziconotide levels, when detectable, remain constant after many months of IT PRIALT infusion in patients followed for up to 9 months.

Distribution

Ziconotide is about 50% bound to human plasma proteins. The mean CSF volume of distribution (Vd) of ziconotide following IT administration approximates the estimated total CSF volume (140 mL).

Metabolism

Ziconotide is cleaved by endopeptidases and exopeptidases at multiple sites on the peptide. Following passage from the CSF into the systemic circulation during continuous IT administration, ziconotide is expected to be susceptible to proteolytic cleavage by various ubiquitous peptidases/proteases present in most

organs (e.g., kidney, liver, lung muscle, etc.), and thus readily degraded to peptide fragments and their individual constituent free amino acids. Human and animal CSF and blood exhibit minimal hydrolytic activity toward ziconotide *in vitro*. The biological activity of the various expected proteolytic degradation products of ziconotide has not been assessed.

Elimination

Minimal amounts of ziconotide (<1%) were recovered in human urine following IV infusion. The terminal half-life of ziconotide in CSF after an IT administration was around 4.6 hours (range 2.9-6.5 hours). Mean CSF clearance (CL) of ziconotide approximates adult human CSF turnover rate (0.3–0.4 mL/min).

Special populations

No formal studies were conducted to assess the effect of demographic factors (age, race, gender, and weight), renal or hepatic dysfunction, or to assess the effect of concomitant drugs on the pharmacokinetics of ziconotide due to the low systemic exposure of ziconotide following IT administration.

CLINICAL TRIALS

The safety and efficacy of IT PRIALT in the management of severe chronic pain were studied in three double-blind, placebo-controlled, multicenter studies in a total of 457 patients (268 PRIALT, 189 placebo) using two different titration schedules. The slow titration schedule tested dose increases 2-3 times per week with a maximum dose of 19.2 mcg/day (0.8 mcg/hr) at 21 days. The fast titration schedule used daily increases up to a maximum dose of 57.6 mcg/day (2.4 mcg/hr) in 5-6 days. The safety in chronic use was studied in four additional open-label, long–term studies in 977 patients.

A randomized, double-blind, placebo-controlled study was conducted at 39 centers to evaluate the efficacy of IT PRIALT administered using a slow titration schedule in 220 patients with severe chronic pain. Patients were randomized 1:1

between PRIALT (112 patients) and placebo (108 patients). At baseline, 97% of these patients reported that their pain was refractory to treatment including IT morphine, IT bupivacaine (an off-label use for this drug) and/or IT clonidine (an off-label use for this drug) in addition to their systemic analgesics and adjunctive therapy. All IT medications were discontinued over a one to three week period and patients were maintained on a stable regimen of non-IT analgesics including opiates, for at least 7 days prior to randomization. This period was successfully completed by 93% of the patients screened. Dosing with PRIALT was started at 2.4 mcg/day (0.1 mcg/hr) and the dose could be increased by 2.4 mcg/day (0.1 mcg/hr) two to three times/week (minimum titration interval 24 hours) to a maximum dose of 19.2 mcg/day (0.8 mcg/hr). The final mean dose at the end of the trial at 21 days was 6.9 mcg/day (0.29 mcg/hr).

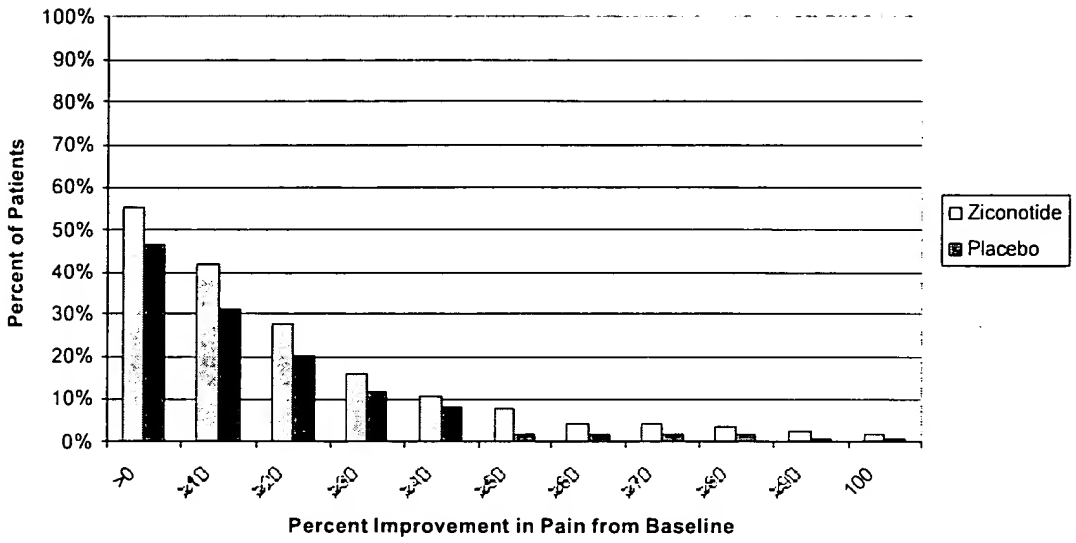
Using a 100 mm Visual Analog Scale of Pain Intensity (VASPI) where 100 mm = worst possible pain, mean baseline pain scores were 81 in both the PRIALT and placebo groups. The primary efficacy variable was the mean percent change in the VASPI score from baseline to day 21. In the intent-to-treat (ITT) efficacy analysis, there was a statistically significant difference between groups in the mean percent change in VASPI score from baseline with the PRIALT group having a 12% mean improvement at Week 3 compared to a 5% mean improvement in the placebo group ($p=0.04$). The 95% confidence interval for the treatment difference (PRIALT – placebo) was 0.4%, 13%.

The effect of IT PRIALT on pain was variable over the time period of treatment for some patients. Some patients had a reduction in VASPI in the first or second week, but did not maintain pain relief by the end of the third week. Other patients, who did not exhibit a reduction in VASPI early in treatment, did have a reduction in VASPI by the third week.

Patients exhibited various degrees of improvement in pain after three weeks of treatment compared with baseline pain assessment. Figure 1 depicts the fraction of patients by their degree of improvement. The figure is cumulative, so that

patients whose change from baseline is, for example, 30%, are also included at every level of improvement below 30%. Patients who did not have a VASPI score recorded at Week 3 (Study days 17-23, inclusive) were assigned 0% improvement. The improvement in the proportion of "responders," defined as having a =30% improvement from baseline in VASPI, was 16% in the PRIALT group compared to 12% in the placebo group, for a net difference of 4%. The use of non IT opioids decreased by 24% in the PRIALT group and by 17% in the placebo group.

Figure 1: Patients Achieving Various Levels of Pain Relief From Baseline to Week 3



INDICATIONS AND USAGE

PRIALT (ziconotide intrathecal infusion) is indicated for the management of severe chronic pain in patients for whom intrathecal (IT) therapy is warranted, and who are intolerant of or refractory to other treatment, such as systemic analgesics, adjunctive therapies or IT morphine.

CONTRAINDICATIONS

PRIALT is contraindicated in patients with a known hyper-sensitivity to ziconotide or any of its formulation components and in patients with any other concomitant treatment or medical condition that would render IT administration hazardous.

Patients with a pre-existing history of psychosis should not be treated with ziconotide.

Contraindications to the use of IT analgesia include conditions such as the presence of infection at the microinfusion injection site, uncontrolled bleeding diathesis, and spinal canal obstruction that impairs circulation of CSF.

WARNINGS

Severe psychiatric symptoms and neurological impairment may occur during treatment with PRIALT. Patients with a pre-existing history of psychosis should not be treated with PRIALT. All patients should be monitored frequently for evidence of cognitive impairment, hallucinations, or changes in mood or consciousness. PRIALT therapy can be interrupted or discontinued abruptly without evidence of withdrawal effects in the event of serious neurological or psychiatric signs or symptoms.

Patients should be cautioned against engaging in hazardous activity requiring complete mental alertness or motor coordination such as operating machinery or driving a motor vehicle during treatment with PRIALT. Patients should also be cautioned about possible combined effects with other CNS-depressant drugs. Dosage adjustments may be necessary when PRIALT is administered with such agents because of the potentially additive effects.

WITHDRAWAL FROM OPIATES

PRIALT is not an opiate and cannot prevent or relieve the symptoms associated with the withdrawal of opiates. To avoid withdrawal syndrome when opiate withdrawal is necessary, patients must NOT be abruptly withdrawn from opiates. For patients being withdrawn from IT opiates, the IT opiate infusion should be gradually tapered over a few weeks and replaced with a pharmacologically equivalent dose of oral opiates. PRIALT does not interact with opiate receptors and does not potentiate opiate-induced respiratory depression.

PRECAUTIONS

General

MENINGITIS AND OTHER INFECTIONS

Meningitis can occur due to inadvertent contamination of the microinfusion device and other means such as CSF seeding due to hematogenous or direct spread from an infected pump pocket or catheter tract. While meningitis is rare with an internal microinfusion device and surgically-implanted catheter, the incidence increases substantially with external devices. In the 1254 patients in PRIALT clinical trials with an exposure of 662 patient-years, meningitis occurred at 3% (40 cases) in the PRIALT group using either internal or external microinfusion devices and 1% (1 case) in the placebo group with an exposure of only 5 patient-years. The risk of meningitis with external microinfusion devices and catheters was higher with 93% cases (38/41) occurring with external infusion systems (37 PRIALT, 1 placebo).

Patients, caregivers, and healthcare providers must be particularly vigilant for the signs and symptoms of meningitis, including but not limited to fever, headache, stiff neck, altered mental status (e.g., lethargy, confusion, disorientation), nausea or vomiting, and occasionally seizures. Serious infection or meningitis can occur within 24 hours of a breach in sterility such as a disconnected catheter, the most common cause of meningitis with external microinfusion devices. The patient and health care provider should be familiar with the handling of the external microinfusion device and care of the catheter skin exit site at risk of infection. Strict aseptic procedures must be used during the preparation of the PRIALT solution or refilling of the microinfusion device to prevent accidental introduction of any contaminants or other environmental pathogens into the reservoir. In suspected cases (especially in immuno-compromised patients) or in confirmed cases of meningitis, CSF cultures must be obtained and appropriate antibiotic therapy must be promptly instituted. Treatment of meningitis usually requires removal of the microinfusion system, catheter, and any other foreign body materials within the IT space and therefore discontinuation of PRIALT therapy.

COGNITIVE AND NEUROPSYCHIATRIC ADVERSE EVENTS

Use of PRIALT has been associated with CNS-related adverse events, including psychiatric symptoms, cognitive impairment, and decreased alertness/unresponsiveness. For the 1254 patients treated, the following cognitive adverse event rates were reported: confusion (33%), memory impairment (22%), speech disorder (14%), aphasia (12%), thinking abnormal (8%), and amnesia (1%). Cognitive impairment may appear gradually after several weeks of treatment. The PRIALT dose should be reduced or discontinued if signs or symptoms of cognitive impairment develop, but other contributing causes should also be considered. The various cognitive effects of PRIALT are generally reversible within 2 weeks after drug discontinuation. The medians for time to reversal of the individual cognitive effects ranged from 3 to 15 days. The elderly (≥65 years of age) are at higher risk for confusion. (See GERIATRIC USE.)

In placebo-controlled trials, there was a higher incidence of suicide, suicide attempts and suicide ideations in PRIALT treated patients (N=3) than in the placebo group (N=1). The incidence was 0.10/patient year for placebo patients and 0.27/patient year for PRIALT patients.

Events of acute psychiatric disturbances such as hallucinations (12%), paranoid reactions (3%), hostility (2%), delirium (2%), psychosis (1%), and manic reactions (0.4%) have been reported in patients treated with PRIALT. Patients with pretreatment psychiatric disorders may be at an increased risk. PRIALT may cause or worsen depression with the risk of suicide in susceptible patients. If appropriate, management of psychiatric complications should include discontinuation of PRIALT, treatment with psychotherapeutic agents if appropriate, and/or short-term hospitalization. Before drug is re-initiated, careful evaluation must be performed on an individual basis.

REDUCED LEVEL OF CONSCIOUSNESS

Patients have become unresponsive or stuporous while receiving PRIALT. The incidence of unresponsiveness or stupor in clinical trials was 2%. During these episodes, the patient sometimes appears to be conscious and breathing is not depressed. If reduced levels of consciousness occur, PRIALT should be discontinued until the event resolves, and other etiologies (e.g., meningitis) should be considered. There is no known pharmacologic antagonist for this effect. Patients taking concomitant antiepileptics, neuroleptics, sedatives, or diuretics may be at higher risk of depressed levels of consciousness. If altered consciousness occurs, other CNS depressant drugs should also be discontinued as clinically appropriate.

ELEVATION OF SERUM CREATINE KINASE (CK-MM)

In clinical studies (mostly open label), 40% of patients had serum creatine kinase (CK) levels above the upper limit of normal, and 11% had CK levels that were ≥ 3 X ULN. In cases where CK was fractionated, only the muscle isoenzyme (MM) was elevated. The time to occurrence was sporadic, but the greatest incidence of CK elevation was during the first two months of treatment. Elevated CKs were more often seen in males, in patients who were being treated with anti-depressants or anti-epileptics, and in patients treated with IT morphine. Most patients who experienced elevations in CK, even for prolonged periods of time, did not have limiting side effects. However, one case of symptomatic myopathy with EMG findings, and two cases of acute renal failure associated with rhabdomyolysis and extreme CK elevations (17,000–27,000 IU/L) have been reported.

Therefore, it is recommended that physicians monitor serum CK in patients undergoing treatment with PRIALT periodically (e.g., every other week for the first month and monthly as appropriate thereafter). Patients should be clinically evaluated and CK measurements obtained in the setting of new neuromuscular symptoms (e.g., myalgias, myasthenia, muscle cramps, asthenia) or a reduction in physical activity. Should these symptoms continue and CK levels remain

elevated or continue to rise, it is recommended that the physician consider PRIALT dose reduction or discontinuation.

INFORMATION FOR PATIENTS

Patients should be cautioned against engaging in hazardous activity requiring complete mental alertness or motor coordination such as operating machinery or driving a motor vehicle during treatment with PRIALT Patients should also be cautioned about possible combined effects with other CNS-depressant drugs. Dosage adjustments may be necessary when PRIALT is administered with such agents because of the potentially additive effects. The physician should be contacted if the patient experiences new or worsening muscle pain, soreness, weakness with or without darkened urine.

PATIENTS AND THEIR CAREGIVERS SHOULD BE INSTRUCTED TO CONTACT A PHYSICIAN IMMEDIATELY IF THE PATIENT HAS

- A change in mental status (e.g., lethargy, confusion, disorientation, decreased alertness)
- A change in mood, perception (hallucinations, including unusual tactile sensations in the oral cavity)
- Symptoms of depression or suicidal ideation
- Nausea, vomiting, seizures, fever, headache, and/or stiff neck, as these may be symptoms of developing meningitis

LABORATORY TESTS

In clinical studies (mostly open label), up to 40% of patients had serum creatine kinase (CK) levels above the upper limit of normal, and 11% had CK levels that were ≥ 3-times the upper limit of normal (see Elevation of Serum Creatine Kinase). Most cases of CK elevation were not associated with muscle

weakness, however one case of myopathy with EMG findings, and two cases of acute renal failure associated with rhabdomyolysis and extreme CK elevations (17,000–27,000 IU/L) were reported..

DRUG INTERACTIONS

Formal PK drug-drug interaction studies have not been performed with PRIALT. As ziconotide is a peptide, it is expected to be completely degraded by endopeptidases and exopeptidases (Phase I hydrolytic enzymes) widely located throughout the body, and not by other Phase I biotransformation processes (including the cytochrome P450 system) or by Phase II conjugation reactions. Thus, IT administration, low plasma ziconotide concentrations and metabolism by ubiquitous peptidases make metabolic interactions of other drugs with ziconotide unlikely. Further, as ziconotide is not highly bound in plasma (approximately 50%) and has low plasma exposure following IT administration, clinically relevant plasma protein displacement reactions involving ziconotide and co-administered medications are unlikely.

Over 90% of patients treated with IT PRIALT used systemic opiates and in the slow titration study, 98% of patients received opioids.

Combination of PRIALT with intrathecal opiates has not been studied in placebo-controlled clinical trials and is not recommended.

Interaction with CNS Depressants

Almost all patients in the PRIALT clinical trials received concomitant non-IT medication. Of the 1254 patients treated, most received several concomitant drugs including antidepressants (66%), anxiolytics (52%), antiepileptics (47%), neuroleptics (46%), and sedatives (34%). The use of drugs with CNS depressant activities may be associated with an increased incidence of CNS adverse events such as dizziness and confusion (see PRECAUTIONS).

Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity studies have been conducted in animals.

Ziconotide was negative in the *in vitro* bacterial reverse mutation assay, *in vitro* mouse lymphoma assay, *in vivo* mouse micronucleus assay, and in the *in vitro* Syrian hamster embryo (SHE) cell transformation assay.

Ziconotide did not affect male fertility in rats when administered as a continuous intravenous (IV) infusion at a dose of up to 10 mg/kg/day when administered for approximately 8 weeks, including a 28-day pre-mating period, or female fertility at a dose of 3 mg/kg/day when administered for approximately 6 weeks, including a 14-day pre-mating period. Estimated exposures for the male and female rats were approximately 6500-fold and 1700-fold higher, respectively, than the expected exposure resulting from the maximum recommended human daily intrathecal (IT) dose of 0.8 mcg/hr (19.2 mcg/day) based on plasma exposure.

Female fertility in rats was significantly affected following continuous IV infusion at a dose of 10 mg/kg/day. Significant reductions in corpora lutea, implantation sites, and number of live fetuses were observed.

Pregnancy

Pregnancy Category C:

Ziconotide was embryolethal in rats when given as a continuous IV infusion during the major period of organogenesis as evidenced by significant increases in post-implantation loss because of an absence or a reduced number of live fetuses. Estimated exposure for embryolethality in the rat was approximately 700-fold above the expected exposure resulting from the maximum recommended human daily intrathecal (IT) dose of 0.8 mcg/hr (19.2 mcg/day). Ziconotide was not teratogenic in female rats when given as a continuous IV infusion at doses up to 30 mg/kg/day or in female rabbits up to 5 mg/kg/day during the major period of organ development. Estimated exposures in the female rat and rabbit were approximately 26,000-fold and 940-fold higher than

the expected exposure resulting from the maximum recommended human daily intrathecal (IT) dose of 0.8 mcg/hr (19.2 mcg/day) based on plasma exposure. Maternal toxicity in the rat and rabbit, as evidenced by decreased body weight gain and food consumption, was present at all dose levels. Maternal toxicity in the rat led to reduced fetal weights and transient, delayed ossification of the pubic bones at doses =15 mg/kg/day which is approximately 8900-fold higher than the expected exposure resulting from the maximum recommended human daily IT dose of 0.8 mcg/hr (19.2 mcg/day) based on plasma exposure. The no observable adverse effect level (NOAEL) for embryo-fetal development in rats was 0.5 mg/kg/day and in rabbits was 5 mg/kg/day. Estimated NOAEL exposures in the rat and rabbit were approximately 400-fold and 940-fold higher than the expected exposure resulting from the maximum recommended human daily IT dose of 0.8 mcg/hr (19.2 mcg/day) based on plasma exposure.

In a pre- and post-natal study in rats, ziconotide given as a continuous IV infusion did not affect pup development or reproductive performance up to a dose of 10 mg/kg/day, which is approximately 3800-fold higher than the expected exposure resulting from the maximum recommended human daily intrathecal (IT) dose of 0.8 mcg/hr (19.2 mcg/day) based on plasma exposure. Maternal toxicity as evidenced by clinical observations, and decreases in body weight gain and food consumption were observed at all doses.

No adequate and well-controlled studies have been conducted in pregnant women. Because animal studies are not always predictive of human response, PRIALT should be used during pregnancy only if the potential benefit justifies risk to the fetus.

Labor and Delivery

The effect of PRIALT on labor and delivery in humans is not known.

Nursing Mothers

It is not known whether PRIALT is excreted in human breast milk. Because many drugs are excreted in human milk, and because of the potential for serious

adverse reactions in nursing infants from PRIALT, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

Geriatric Use

Of the total number of subjects in clinical studies of PRIALT, 22% were 65 and over, while 7% were 75 and over. In all trials, there was a higher incidence of confusion in older patients (42% for ≥65 year old versus 29% for <65 year old subgroups). Other reported clinical experience has not identified differences in responses between the elderly and younger patients. In general, the dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal or cardiac function, and of concomitant disease or other drug therapy.

Hepatic and Renal Impairment

Formal PK studies were not conducted in patients with hepatic or renal impairment.

ADVERSE REACTIONS

The safety of IT PRIALT administered as a continuous infusion has been evaluated in 1254 patients participating in acute and severe chronic pain trials. The duration of treatment has ranged from a one-hour IT infusion to treatment lasting for more than 7.5 years. The mean duration of treatment was 193 days with 173 patients (14%) treated for at least 1 year. The average final dose was 17.6 mcg/day (0.73 mcg/hr).

The most frequently reported adverse events (=25%) in the 1254 patients (662 patient years) in clinical trials were dizziness, nausea, confusion, headache, somnolence, nystagmus, asthenia, and pain. Serious adverse events and discontinuation of PRIALT for adverse events are less frequent when the drug is

slowly titrated over 21 days, than with a faster titration schedule. (See CLINICAL TRIALS and DOSAGE and ADMINISTRATION.)

Table 2 summarizes the treatment-emergent adverse events with a frequency of 5% or greater in the PRIALT-treated group from the one placebo-controlled trial using the slow titration schedule in patients with severe chronic pain. All events reported during the initial placebo-controlled period of the studies (21 days in the slow titration schedule) are tabulated, regardless of relationship to PRIALT.

Table 2. Incidence of Treatment-Emergent Adverse Events in Slow Titration Placebo-Controlled Trial by Percent (Events That Occurred in ≥ 5% of patients and more commonly with PRIALT than with placebo)

	PRIALT N=112	**Placebo** N=108
	Percentages of Patients	
Any AE	93	82
Body as a Whole	57	42
Asthenia	22	12
Headache	15	12
Pain	11	7
Fever	7	3
Digestive	60	51
Nausea	41	31
Diarrhea	19	17
Vomiting	15	13
Anorexia	10	5
Nervous System	81	51
Dizziness	47	13
Somnolence	22	15
Confusion	18	5
Ataxia	16	2
Abnormal Gait	15	2
Memory Impairment	12	1
Hypertonia	11	5
Anxiety	9	5
Speech Disorder	9	2
Aphasia	8	1
Nystagmus	8	0
Dysesthesia	7	2
Hallucinations	7	0
Nervousness	7	4
Paresthesia	7	3
Vertigo	7	0
Special Senses	20	11
Abnormal Vision	10	4
Urogenital	22	12
Urinary Retention	9	0

The following adverse events assessed as related to PRIALT have been reported in 2% or greater of patients participating in the clinical studies. (COSTART terms, by body system):

BODY AS A WHOLE: abdominal pain, accidental injury, asthenia, back pain, catheter complication, catheter site pain, cellulitis, chest pain, chills, fever, flu syndrome, headache, infection, malaise, neck pain, neck rigidity, pain, pump site complication, pump site mass, pump site pain, viral infection. CARDIOVASCULAR SYSTEM: hypertension, hypotension, postural hypotension, syncope, tachycardia, vasodilation. DIGESTIVE SYSTEM: anorexia, constipation, diarrhea, dyspepsia, gastrointestinal disorder, nausea, nausea and vomiting, vomiting. HEMIC AND LYMPHATIC SYSTEM: anemia, ecchymosis. METABOLIC AND NUTRITIONAL DISORDER: creatinine phosphokinase increased, dehydration, edema, hypokalemia, peripheral edema, weight loss. MUSCULOSKELETAL SYSTEM: arthralgia, arthritis, leg cramps, myalgia, myasthenia. NERVOUS SYSTEM: abnormal dreams, abnormal gait, agitation, anxiety, aphasia, ataxia, cerebrospinal fluid abnormal, confusion, depression, difficulty concentrating, dizziness, dry mouth, dysesthesia, emotional lability, hostility, hyperesthesia, hypertonia, incoordination, insomnia, memory impairment, mental slowing, meningitis, nervousness, neuralgia, nystagmus, paranoid reaction, paresthesia, reflexes decreased, somnolence, speech disorder, stupor, thinking abnormal, tremor, twitching, vertigo. RESPIRATORY SYSTEM: bronchitis, cough increased, dyspnea, lung disorder, pharyngitis, pneumonia, rhinitis, sinusitis. SKIN AND APPENDAGES: cutaneous surgical complication, dry skin, pruritus, rash, skin disorder, sweating. SPECIAL SENSES: abnormal vision, diplopia, photophobia, taste perversion, tinnitus. UROGENITAL SYSTEM: dysuria, urinary incontinence, urinary retention, urinary tract infection, urination impaired.

At less than 2%, the following events were assessed by the clinical investigators as related to PRIALT: acute kidney failure, atrial fibrillation, cerebrovascular accident, electrocardiogram abnormal, grand mal convulsion, meningitis, myoclonus, psychosis, respiratory distress, rhabdomyolysis, sepsis, and suicidal ideations. Rare instances of fatal aspiration pneumonia and suicide were reported (<1%).

OVERDOSAGE

The maximum recommended IT PRIALT dose is 19.2 mcg/day. The maximum IT dose of PRIALT in clinical trials was 912 mcg/day. In some patients who received IT doses greater than the maximum recommended dose, exaggerated pharmacological effects (e.g., ataxia, nystagmus, dizziness, stupor, unresponsiveness, spinal myoclonus, confusion, sedation, hypotension, word-finding difficulties, garbled speech, nausea, and vomiting) were observed. There was no indication of respiratory depression. Overdoses may occur due to pump programming errors or incorrect drug concentration preparations. In these cases, patients were observed and ziconotide was either temporarily discontinued or permanently withdrawn. Most patients recovered within 24 hours after withdrawal of drug. In the event of an IT overdose, elimination of ziconotide from CSF would be expected to remain constant (CSF $t_{½}$ = 4.6 hours). Therefore within 24 hours of stopping therapy, the ziconotide CSF concentration should be less than 5% of peak levels.

There is no known antidote to ziconotide. General medical supportive measures should be administered to patients who receive an overdose until the exaggerated pharmacological effects of the drug have resolved. Treatment for an overdose is hospitalization, when needed, and symptom related supportive care. Ziconotide does not bind to opiate receptors and its pharmacological effects are not blocked by opioid antagonists.

In the event of an inadvertent intravenous or epidural administration, adverse events could include hypotension, which can be treated with a recumbent posture and blood pressure support as required. The half-life of PRIALT in serum is 1.3 hours.

DOSAGE AND ADMINISTRATION

IT PRIALT should be initiated at no more than 2.4 mcg/day (0.1 mcg/hr) and titrated to patient response. Doses may be titrated upward by up to 2.4 mcg/day (0.1 mcg/hr) at intervals of no more than 2-3 times per week, up to a recommended maximum of 19.2 mcg/day (0.8 mcg/hr) by Day 21. Dose

increases in increments of less than 2.4 mcg/day (0.1 mcg/hr) and increases in dose less frequently than 2-3 times per week may be used. For each dose titration, assess the dosing requirements and adjust the pump infusion flow rate as required to achieve the new dosing. Controlled studies of pain relief have not been conducted for longer than 3 weeks duration, although 977 patients have been treated with IT PRIALT in long-term open-label trials.

The dose of IT PRIALT should be adjusted according to the patient's severity of pain, their response to therapy and the occurrence of adverse events. The effective dose of PRIALT for analgesia is variable. The average dose level at the end of the 21-day titration used in the slow titration clinical trial (SEE CLINICAL TRIALS) was 6.9 mcg/day (0.29 mcg/hr) and the maximum dose was 19.2 mcg/day (0.8 mcg/hr) on Day 21. Due to the frequency of adverse events, 19.2 mcg/day (0.8 mcg/hr) is the maximum recommended dose.

Because of the lower incidence of serious adverse events and discontinuations for adverse events associated with the slower titration (see ADVERSE REACTIONS), a faster titration schedule should only be used if there is an urgent need for analgesia that outweighs the risk to patient safety.

In clinical trials, no rebound or other adverse events related to discontinuation of PRIALT were noted, although treatment was almost always discontinued abruptly.

Vials of PRIALT should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Administration

PRIALT should be administered intrathecally (IT) by or under the direction of a physician experienced in the technique of IT administration and who is familiar with the drug and device labeling. PRIALT is not intended for intravenous administration.

PRIALT is intended for IT delivery using a programmable implanted variable-rate microinfusion device or an external microinfusion device and catheter (see PRECAUTIONS-Meningitis and Other Infections). Refer to the manufacturer's manual for specific instructions and precautions for programming the microinfusion device and/or refilling the reservoir.

PRIALT is used for therapy undiluted (25 mcg/mL in 20mL vial) or diluted (100 mcg/mL in 1, 2 or 5 mL vials). Diluted PRIALT is prepared with 0.9% Sodium Chloride Injection, USP (preservative free) using aseptic procedures to the desired concentration prior to placement in the microinfusion pump. The 100 mcg/mL formulation may be administered undiluted once an appropriate dose has been established. SALINE SOLUTIONS CONTAINING PRESERVATIVES ARE NOT APPROPRIATE FOR IT DRUG ADMINISTRATION AND SHOULD NOT BE USED. Refrigerate but do not freeze all PRIALT solutions after preparation and begin infusion within 24 hours. Discard any PRIALT solution with observed particulate matter or discoloration and any unused portion left in the vial.

Medtronic SynchroMed EL or SynchroMed II Infusion System (SEE PRECAUTIONS-Meningitis and Other Infections)

Refer to the manufacturer's manuals for specific instructions and precautions for performing a reservoir rinse, initial filling, refilling the reservoir, and programming.

Instructions for Use of PRIALT with Pump

1. Naïve Pump Priming (i.e., first time use with PRIALT)

Only the undiluted 25 mcg/mL formulation should be used for naïve pump priming. Rinse the internal surfaces of the pump with 2 mL of PRIALT at 25 mcg/mL. Repeat twice for a total of three rinses.

2. Initial Pump Fill

Only the undiluted 25 mcg/mL formulation should be used for initial pump fill. Fill the naïve pump after priming as above with the appropriate volume of

PRIALT at 25 mcg/mL. Begin dosing at a delivery rate no higher than 2.4 mcg/day (0.1 mcg/hr). In a naïve pump, PRIALT is lost due to two factors that do not occur upon subsequent refills: adsorption on internal device surfaces, such as the titanium, and by dilution in the residual space of the device. Consequently, the pump reservoir should be refilled with PRIALT within 14 days of the initial fill to ensure appropriate dose administration.

3. Pump Refills

For subsequent pump refills, fill the pump at least every 40 days if PRIALT is used diluted. For undiluted PRIALT, fill the pump at least every 60 days. To ensure aseptic transfer of PRIALT into the device, it is recommended that the Medtronic refill kit be used. The pump contents should be emptied prior to refill with PRIALT.

If the internal infusion system must be surgically replaced while the person is receiving PRIALT, the replacement pump should be rinsed with PRIALT (No. 1 above), and this initial fill solution must be replaced within 14 days (No. 2 above). Subsequent refills should be done at least every 60 days if PRIALT is used undiluted or at least every 40 days if PRIALT is used diluted.

PRIALT (ziconotide intrathecal infusion)	**Initial Fill** Expiry	**Refill** Expiry
25 mcg/mL, undiluted	14 Days	60 Days
100 mcg/mL, undiluted	N/A	60 Days
100 mcg/mL, diluted	N/A	40 Days

Simms Deltec Cadd Micro External Microinfusion Device and Catheter (See PRECAUTIONS-Meningitis and Other Infections).

Refer to the manufacturer's manuals for specific instructions and precautions for performing the initial filling, refilling of the reservoir or replacement of the drug cartridge, and operation. The appropriate external microinfusion device is filled for the first time with PRIALT solution at a concentration of 5 mcg/mL. This solution is prepared by diluting PRIALT with 0.9% Sodium Chloride, USP (preservative free). The flow rate for the external microinfusion device usually starts at 0.02 mL/hr to deliver the initial dose rate of 2.4 mcg/day (0.1 mcg/hr) of PRIALT. Changes in dose rate are made by adjusting the flow rate of the infusion system and/or the concentration of PRIALT solution.

HOW SUPPLIED

PRIALT is supplied as a 25 mcg/mL solution in a single-use 20 mL glass vial and as a 100 mcg/mL solution in single-use glass vials containing 1 mL, 2 mL, or 5 mL of solution. One vial is packaged per carton.

Presentation (NDC)

25 mcg/mL: 20 mL vial (59075-723-10). Only the undiluted 25 mcg/mL formulation should be used for PRIALT naïve pump priming.

100 mcg/mL: 1 mL (59075-720-10)

2 mL (59075-721-10)

5 mL (59075-722-10)

STORAGE

- Refrigerate PRIALT during transit.
- Store PRIALT at 2°C–8°C (36°F–46°F).
- PRIALT, once diluted aseptically with saline, may be stored at 2°C–8°C for 24 hours
- Do NOT freeze PRIALT.
- Protect from light.

Distributed by:
Elan Pharmaceuticals, Inc.
San Diego, CA 92121

(Rx Only Symbol) (ELAN Logo)

6000323 Rev. 12/04

Package Colors
Blue PMS 285
Green PMS 361
Barcode Imprint Black

*BAR CODES ARE FOR POSITION ONLY

Elan Pharmaceutical Version 076A
1 – 7/16 X 1 – 3/16 X 2 – 23/32
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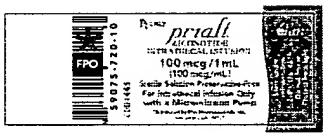

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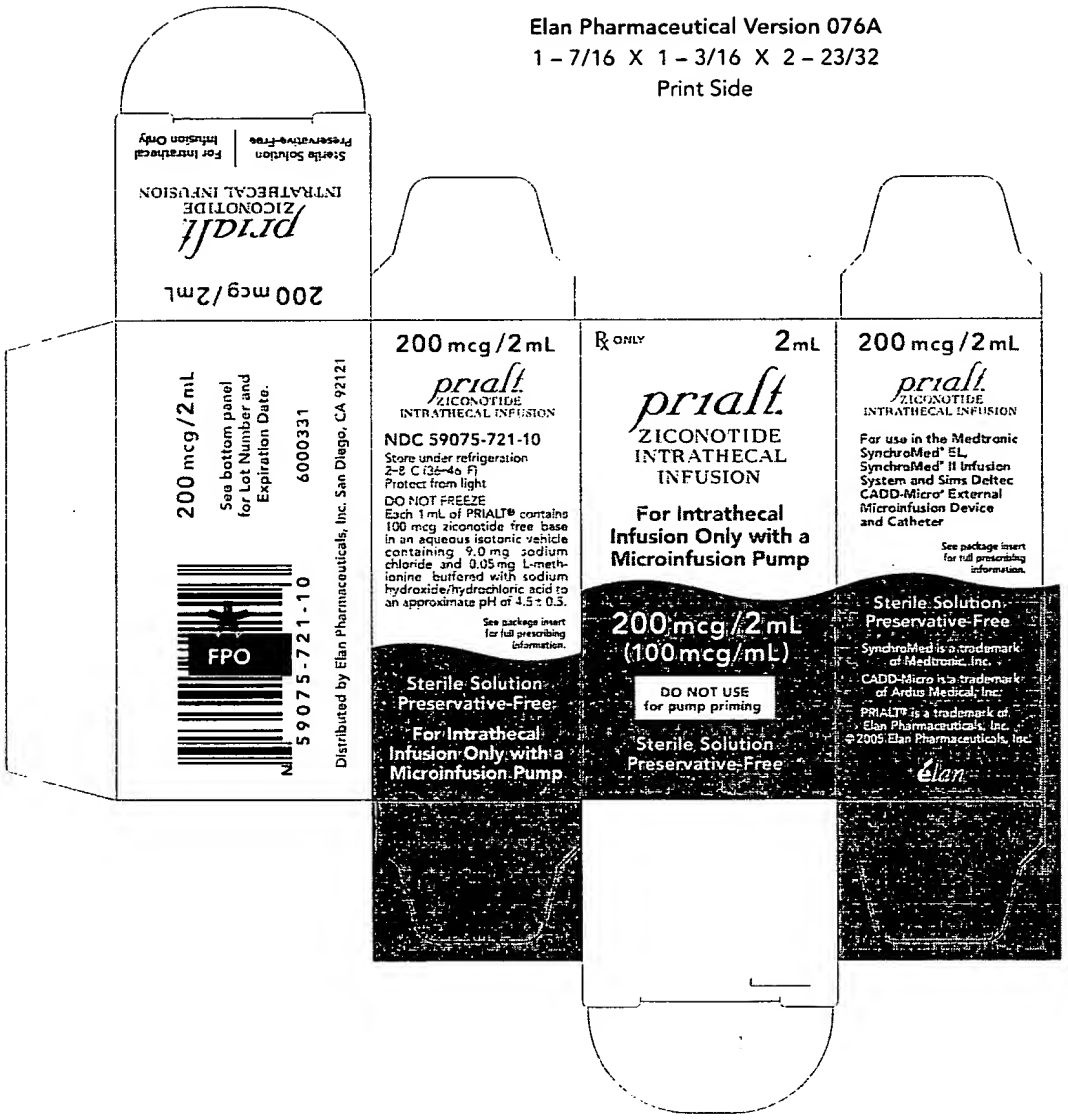

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Package Colors
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Package Colors
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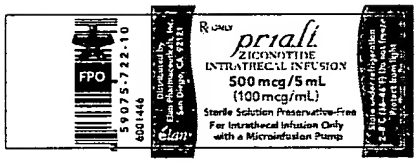

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Package Colors
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Barcode Imprint Black

*BAR CODES ARE FOR POSITION ONLY

Elan Pharmaceutical Version 076A
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Package Colors
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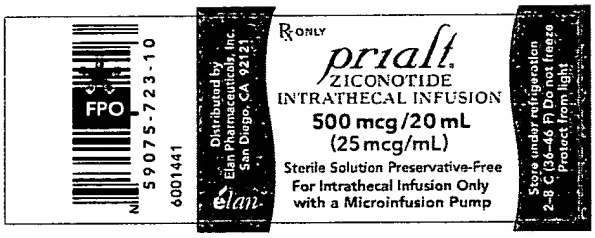

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/s/

Robert Meyer
12/28/04 01:01:00 PM

Attachment D

US005795864A

United States Patent [19]

Amstutz et al.

[11] Patent Number: 5,795,864

[45] Date of Patent: Aug. 18, 1998

[54] **STABLE OMEGA CONOPETIDE FORMULATIONS**

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[21] Appl. No.: **496,847**

[22] Filed: **Jun. 27, 1995**

[51] **Int. Cl.**[6] **A61K 38/00**; C07K 5/00; C07K 7/00

[52] **U.S. Cl.** **514/12**; 530/324

[58] **Field of Search** 530/324; 514/12

[56] **References Cited**

U.S. PATENT DOCUMENTS

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Primary Examiner—Avis M. Davenport

Attorney, Agent, or Firm—Peter J. Dehlinger; Carol A. Stratford

[57] **ABSTRACT**

Disclosed are formulations effective to stabilize omega conotoxin peptide preparations at elevated temperatures. Novel omega conopeptides also form part of the invention.

7 Claims, 12 Drawing Sheets

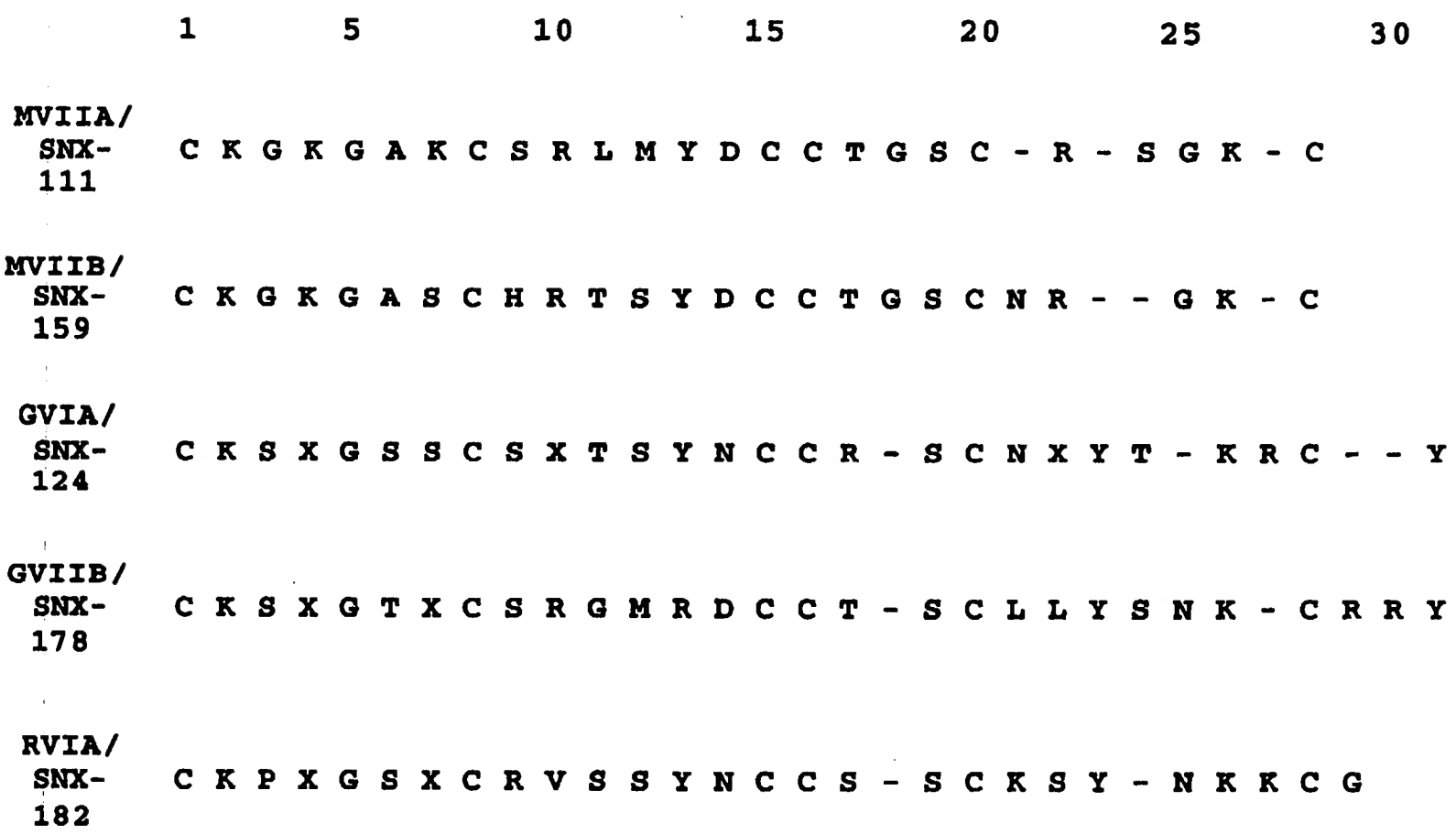
1 5 10 15 20 25 30
MVIIA/ SNX-111
C K G K G A K C S R L M Y D C C T G S C - R - S G K - C
MVIIB/ SNX-159
C K G K G A S C H R T S Y D C C T G S C N R - - G K - C
GVIA/ SNX-124
C K S X G S S C S X T S Y N C C R - S C N X Y T - K R C - - Y
GVIIB/ SNX-178
C K S X G T X C S R G M R D C C T - S C L L Y S N K - C R R Y
RVIA/ SNX-182
C K P X G S X C R V S S Y N C C S - S C K S Y - N K K C G

Fig. 1A

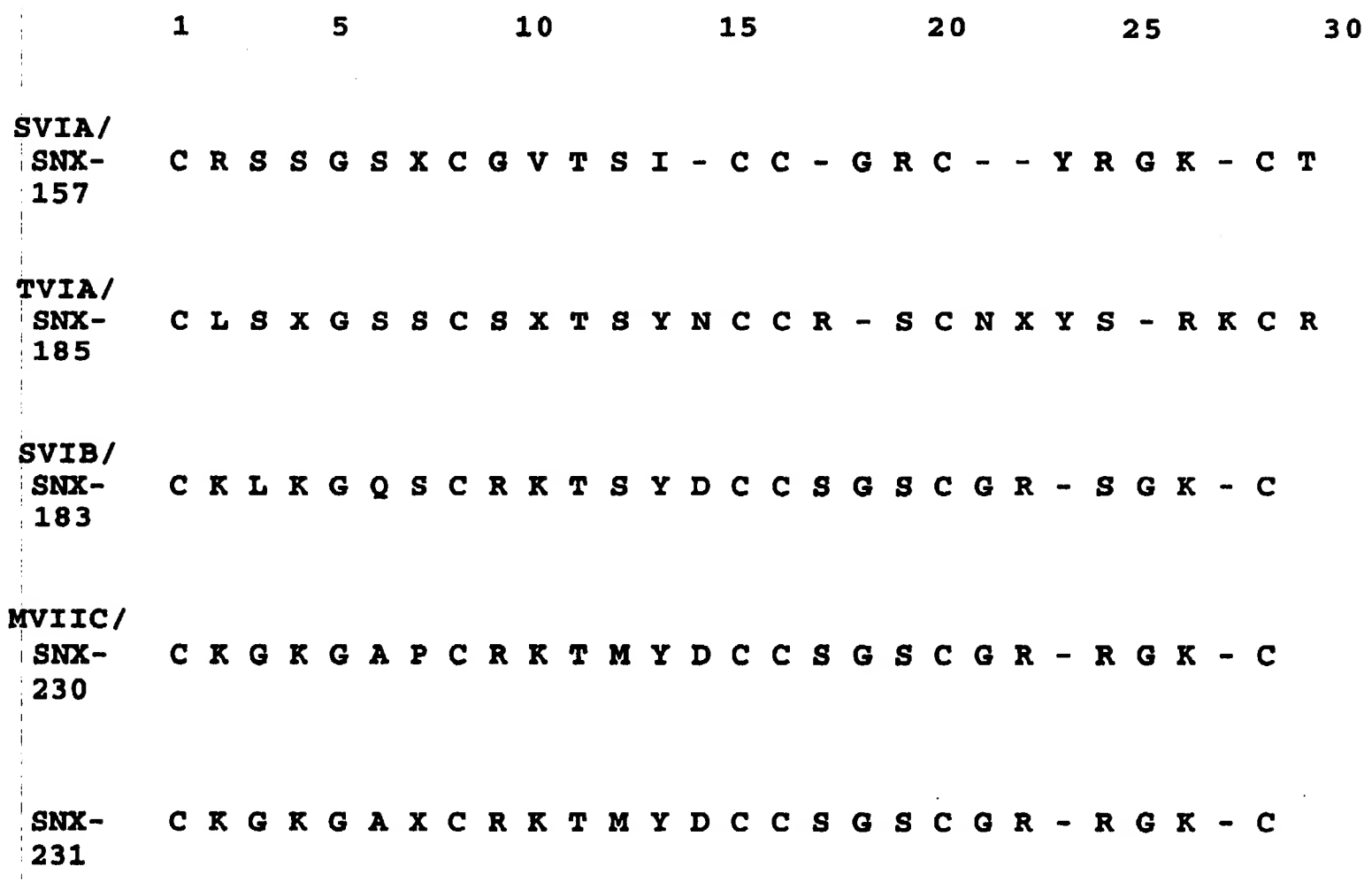
1 5 10 15 20 25 30
SVIA/SNX-157 C R S S G S X C G V T S I - C C - G R C - - Y R G K - C T
TVIA/SNX-185 C L S X G S S C S X T S Y N C C R - S C N X Y S - R K C R
SVIB/SNX-183 C K L K G Q S C R K T S Y D C C S G S C G R - S G K - C
MVIIC/SNX-230 C K G K G A P C R K T M Y D C C S G S C G R - R G K - C
SNX-231 C K G K G A X C R K T M Y D C C S G S C G R - R G K - C

Fig. 1B

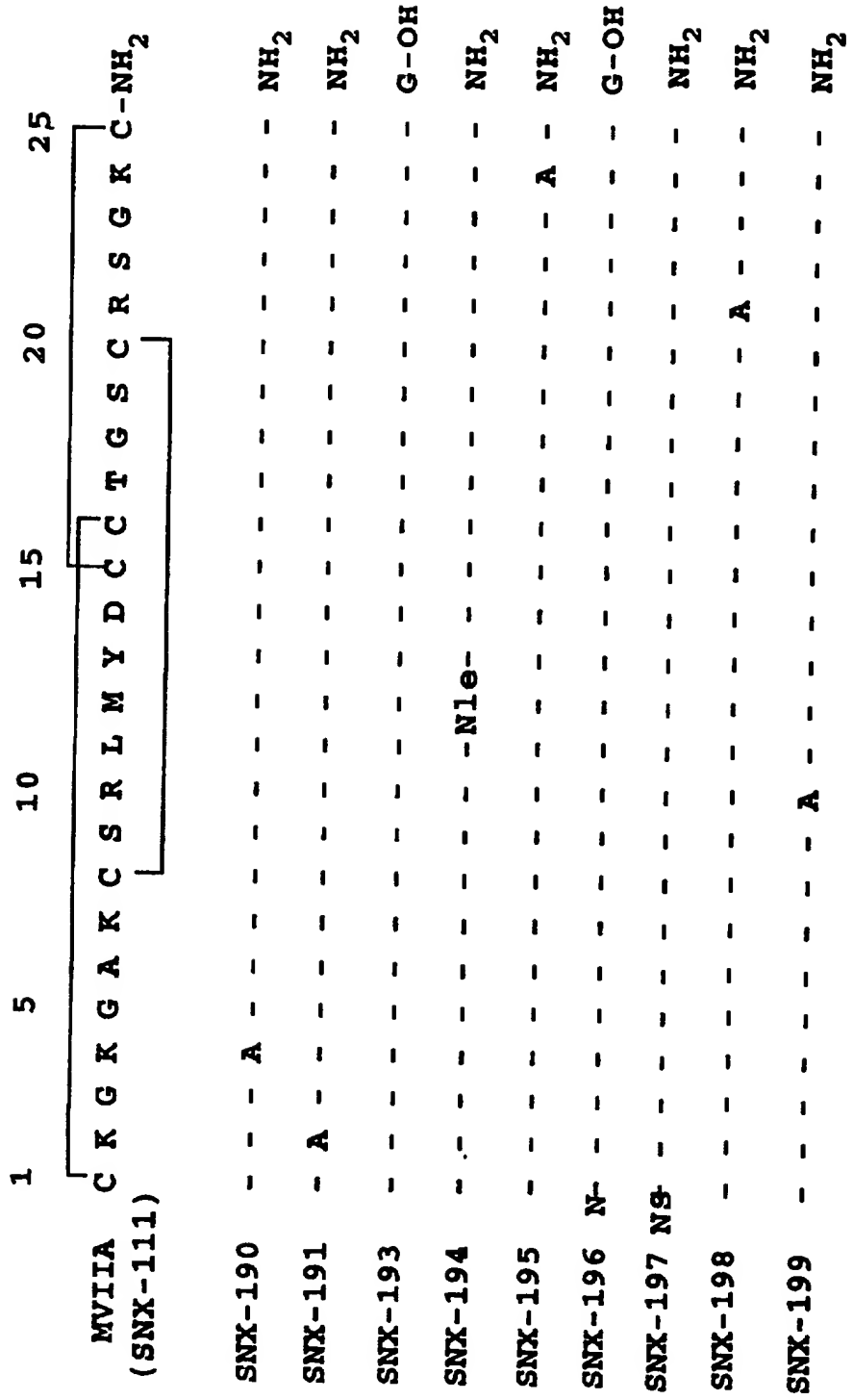
1 5 10 15 20 25
MVIIA C K G K G A K C S R L M Y D C C T G S C R S G K C-NH2
(SNX-111)
SNX-190 - - - A - NH2
SNX-191 - A - NH2
SNX-193 - G-OH
SNX-194 - - - - - - - - - -Nle- - - - - - - - - - - - - NH2
SNX-195 - A - - NH2
SNX-196 N- G-OH
SNX-197 N8- NH2
SNX-198 - A - - - NH2
SNX-199 - - - - - - - - - A - - - - - - - - - - - - - - NH2

Fig. 2A

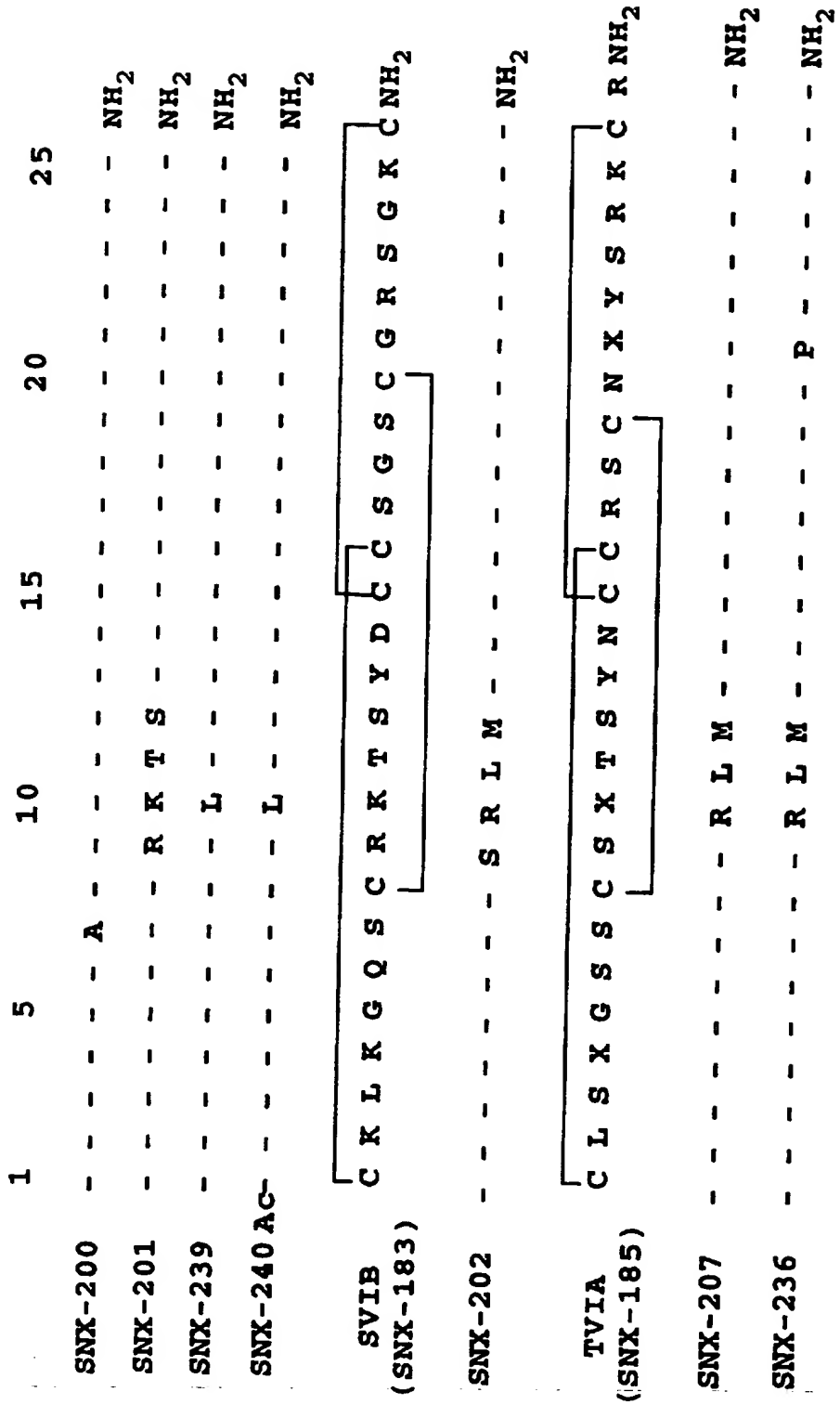
1 5 10 15 20 25
SNX-200 - - - - - A - - - - - - - - - - - - - - - - - NH2
SNX-201 - - - - - - R K T S - - - - - - - - - - - - - NH2
SNX-239 - - - - - - - L - - - - - - - - - - - - - - NH2
SNX-240 Ac- - - - - - - L - - - - - - - - - - - - - - NH2
SVIB (SNX-183) C K L K G Q S C R K T S Y D C C S G S C G R S G G K C NH2
SNX-202 - - - - - - - - - - S R L M - - - - - - - - - - - - - - - NH2
TVIA (SNX-185) C L S X G S S C S X T S Y N C C R S C N X Y S R K C R NH2
SNX-207 - - - - - - - - - - - R L M - - - - - - - - - - - - - - - NH2
SNX-236 - - - - - - - - - - - R L M - - - - - - - - P - - - - - - NH2

Fig. 2B

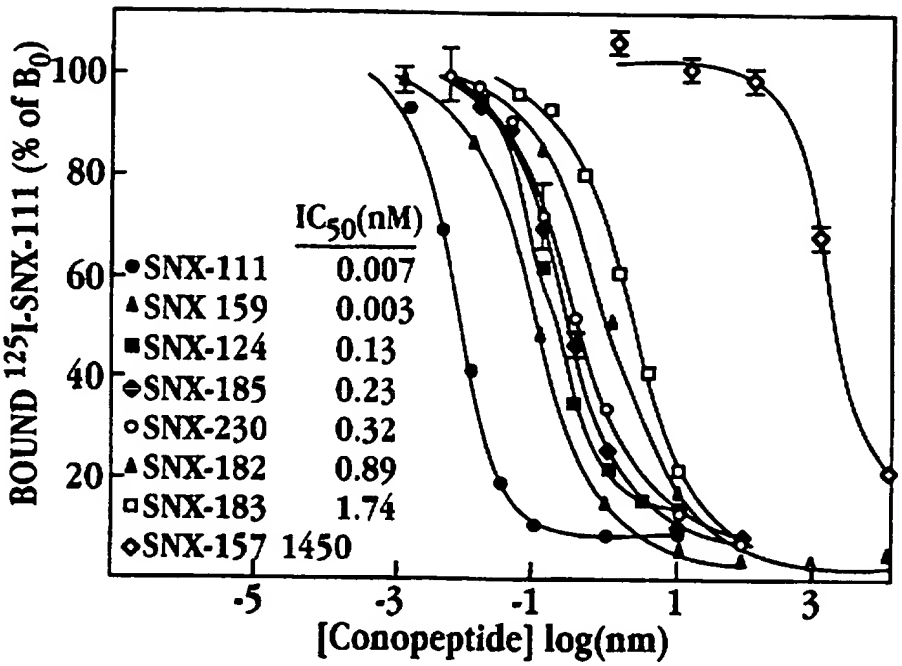
BOUND ^{125}I-SNX-111 (% of B_0)
100
80
60
40
20
IC_{50}(nM)
SNX-111 0.007
SNX 159 0.003
SNX-124 0.13
SNX-185 0.23
SNX-230 0.32
SNX-182 0.89
SNX-183 1.74
SNX-157 1450
-5
-3
-1
1
3
[Conopeptide] log(nm)

Fig. 3A

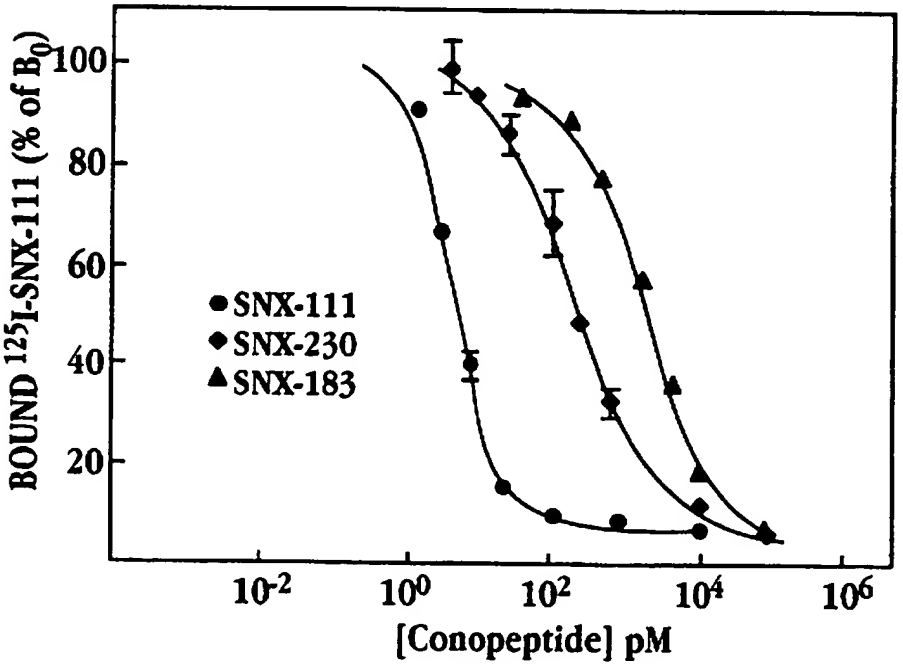
BOUND ^{125}I-SNX-111 (% of B_0)
100
80
60
40
20
SNX-111
SNX-230
SNX-183
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
[Conopeptide] pM

Fig. 3B

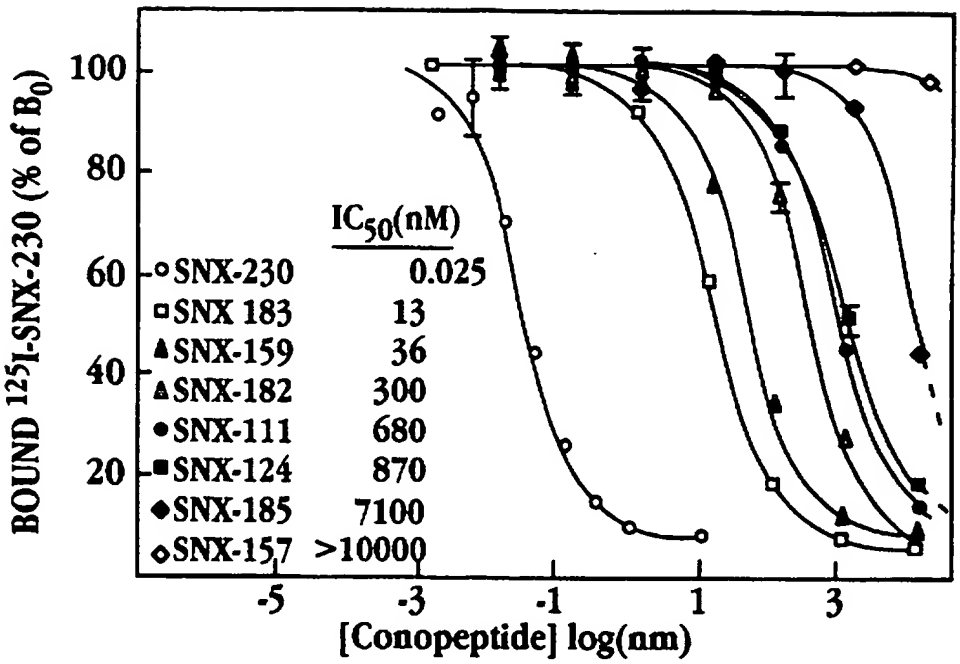
BOUND ^{125}I-SNX-230 (% of B_0)
100
80
60
40
20
IC_{50}(nM)
SNX-230 0.025
SNX 183 13
SNX-159 36
SNX-182 300
SNX-111 680
SNX-124 870
SNX-185 7100
SNX-157 >10000
-5
-3
-1
1
3
[Conopeptide] log(nm)

Fig. 4A

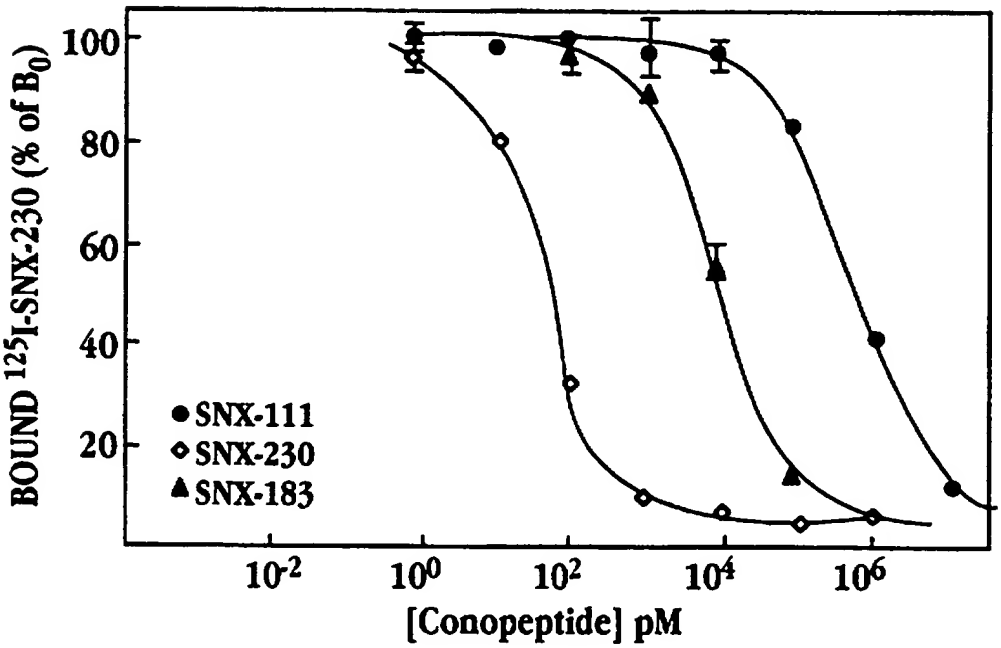
BOUND ^{125}I-SNX-230 (% of B_0)
100
80
60
40
20
SNX-111
SNX-230
SNX-183
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
[Conopeptide] pM

Fig. 4B

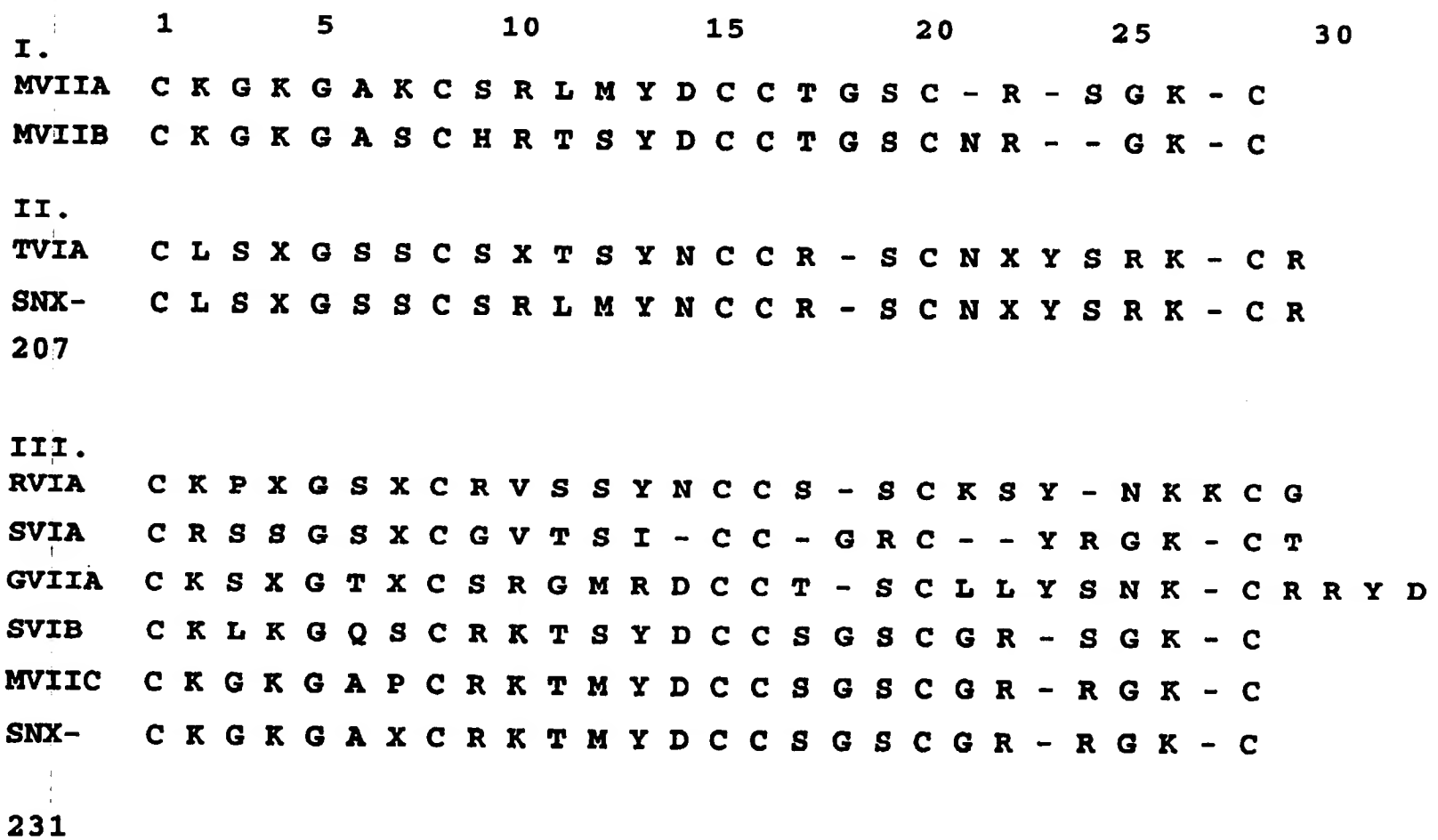
1 5 10 15 20 25 30
I.
MVIIA C K G K G A K C S R L M Y D C C T G S C - R - S G K - C
MVIIB C K G K G A S C H R T S Y D C C T G S C N R - - G K - C
II.
TVIA C L S X G S S C S X T S Y N C C R - S C N X Y S R K - C R
SNX- C L S X G S S C S R L M Y N C C R - S C N X Y S R K - C R
207
III.
RVIA C K P X G S X C R V S S Y N C C S - S C K S Y - N K K C G
SVIA C R S S G S X C G V T S I - C C - G R C - - Y R G K - C T
GVIIA C K S X G T X C S R G M R D C C T - S C L L Y S N K - C R R Y D
SVIB C K L K G Q S C R K T S Y D C C S G S C G R - S G K - C
MVIIC C K G K G A P C R K T M Y D C C S G S C G R - R G K - C
SNX- C K G K G A X C R K T M Y D C C S G S C G R - R G K - C
231

Fig. 5

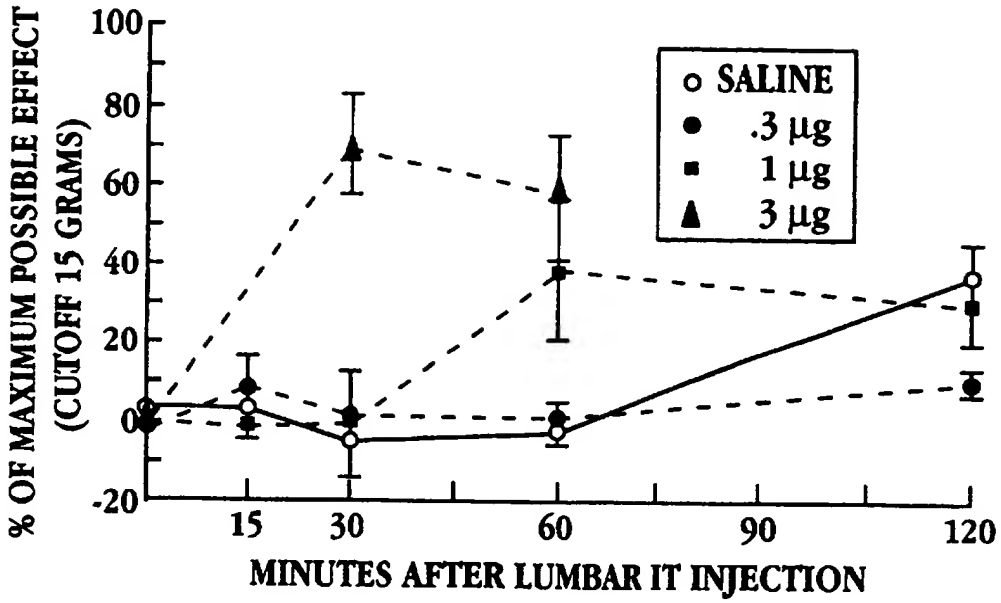
% OF MAXIMUM POSSIBLE EFFECT
(CUTOFF 15 GRAMS)
100
80
60
40
20
0
-20
15
30
60
90
120
MINUTES AFTER LUMBAR IT INJECTION
SALINE
.3 µg
1 µg
3 µg

Fig. 6A

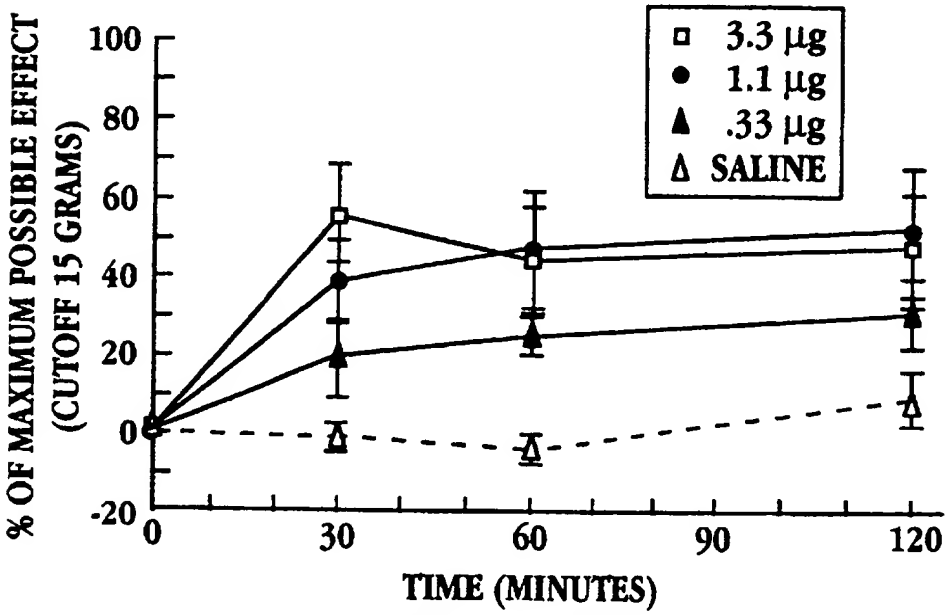
% OF MAXIMUM POSSIBLE EFFECT
(CUTOFF 15 GRAMS)
100
80
60
40
20
0
-20
0
30
60
90
120
TIME (MINUTES)
3.3 µg
1.1 µg
.33 µg
SALINE

Fig. 6B

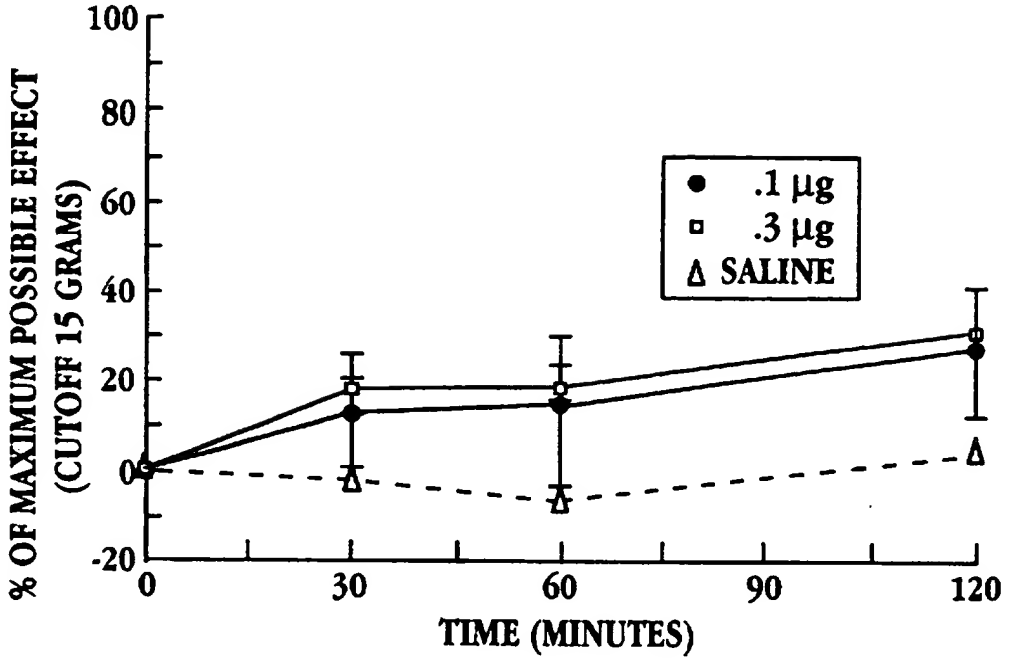
100
80
60
40
20
0
-20
% OF MAXIMUM POSSIBLE EFFECT
(CUTOFF 15 GRAMS)
.1 µg
.3 µg
Δ SALINE
0
30
60
90
120
TIME (MINUTES)

Fig. 6C

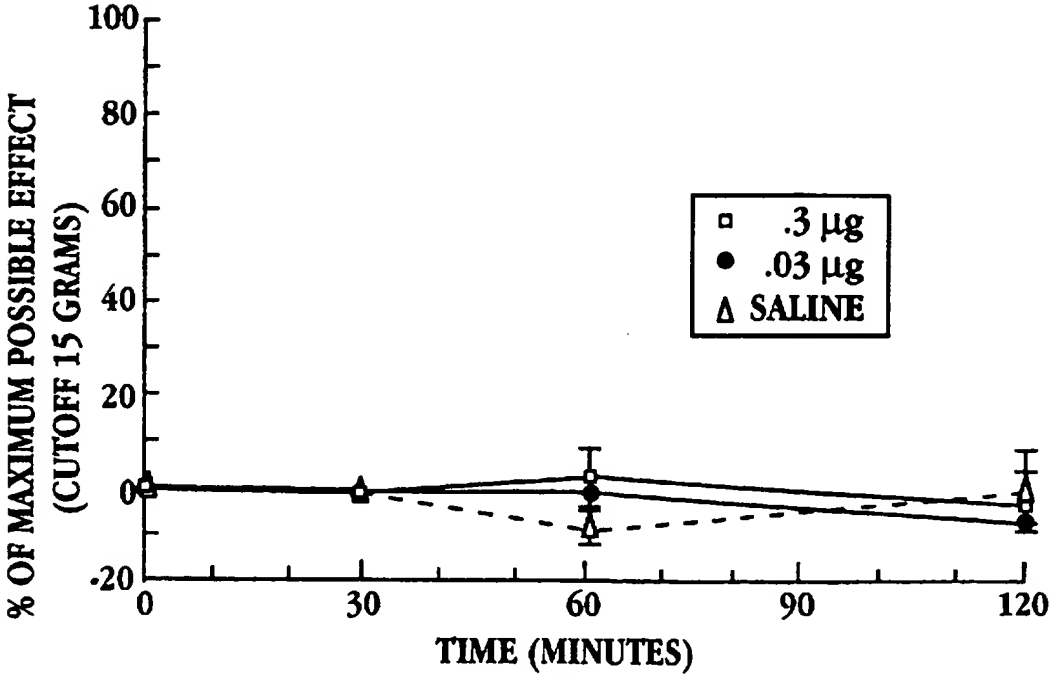
100
80
60
40
20
0
-20
% OF MAXIMUM POSSIBLE EFFECT
(CUTOFF 15 GRAMS)
.3 µg
.03 µg
Δ SALINE
0
30
60
90
120
TIME (MINUTES)

Fig. 6D

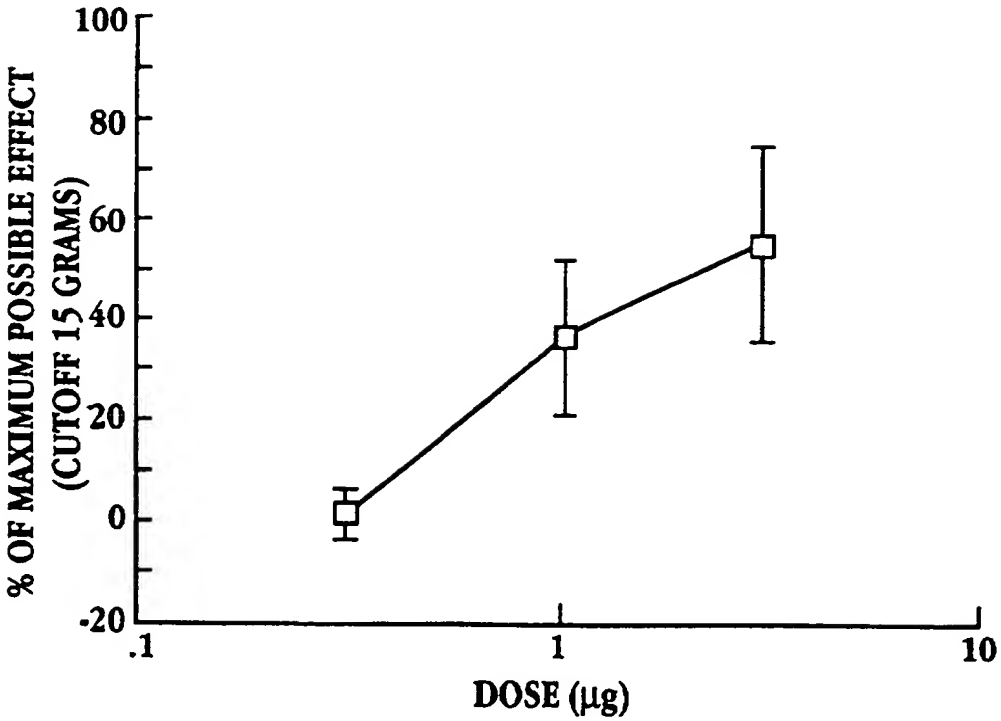
% OF MAXIMUM POSSIBLE EFFECT
(CUTOFF 15 GRAMS)
100
80
60
40
20
0
-20
.1
1
10
DOSE (μg)

Fig. 7A

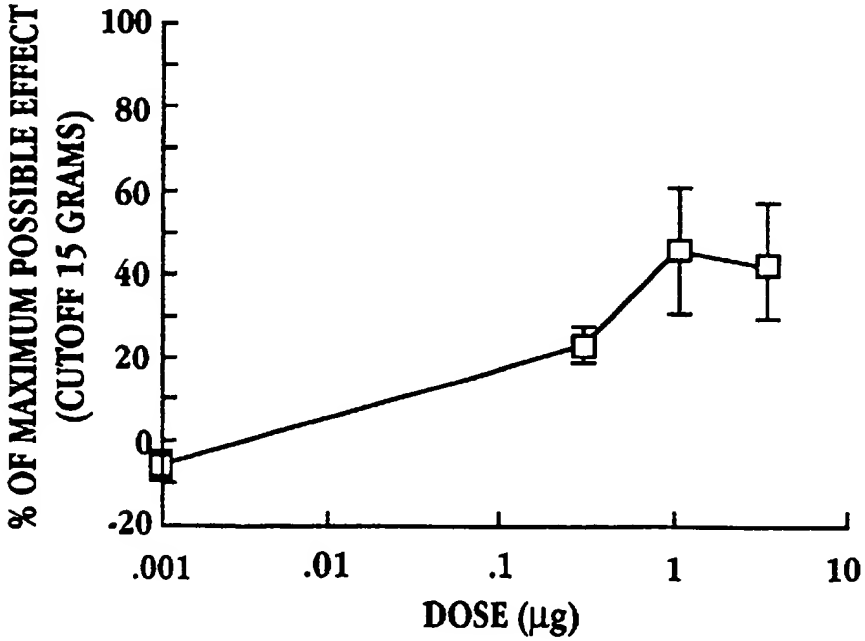
% OF MAXIMUM POSSIBLE EFFECT
(CUTOFF 15 GRAMS)
100
80
60
40
20
0
-20
.001
.01
.1
1
10
DOSE (μg)

Fig. 7B

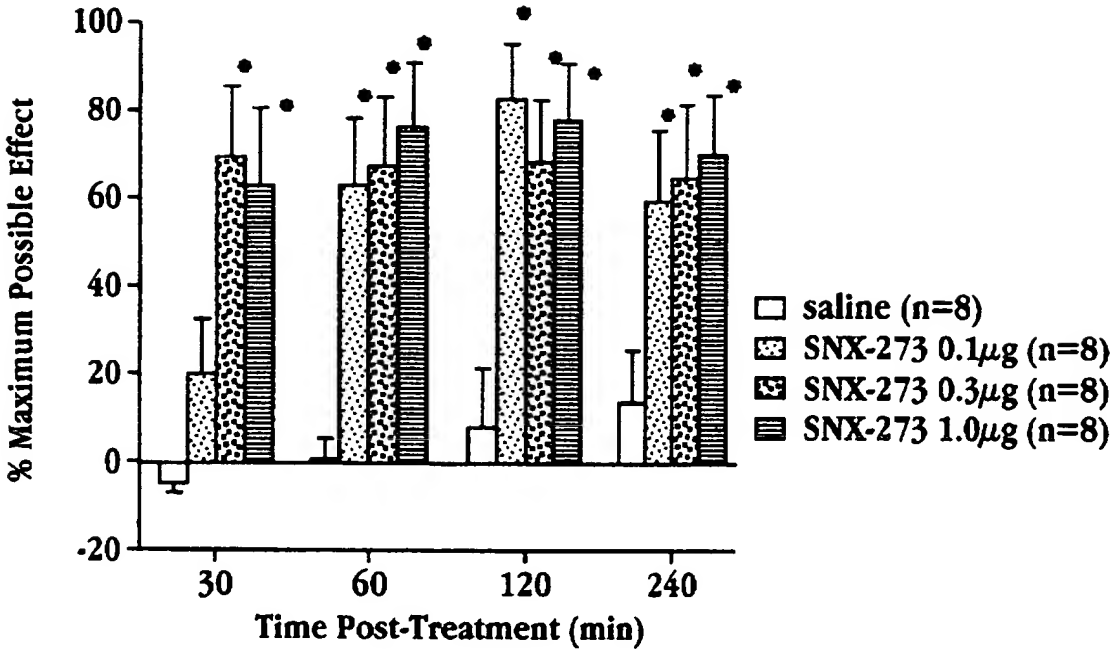
100
80
60
40
20
0
-20
% Maximum Possible Effect
30
60
120
240
Time Post-Treatment (min)
saline (n=8)
SNX-273 0.1μg (n=8)
SNX-273 0.3μg (n=8)
SNX-273 1.0μg (n=8)

Fig. 8A

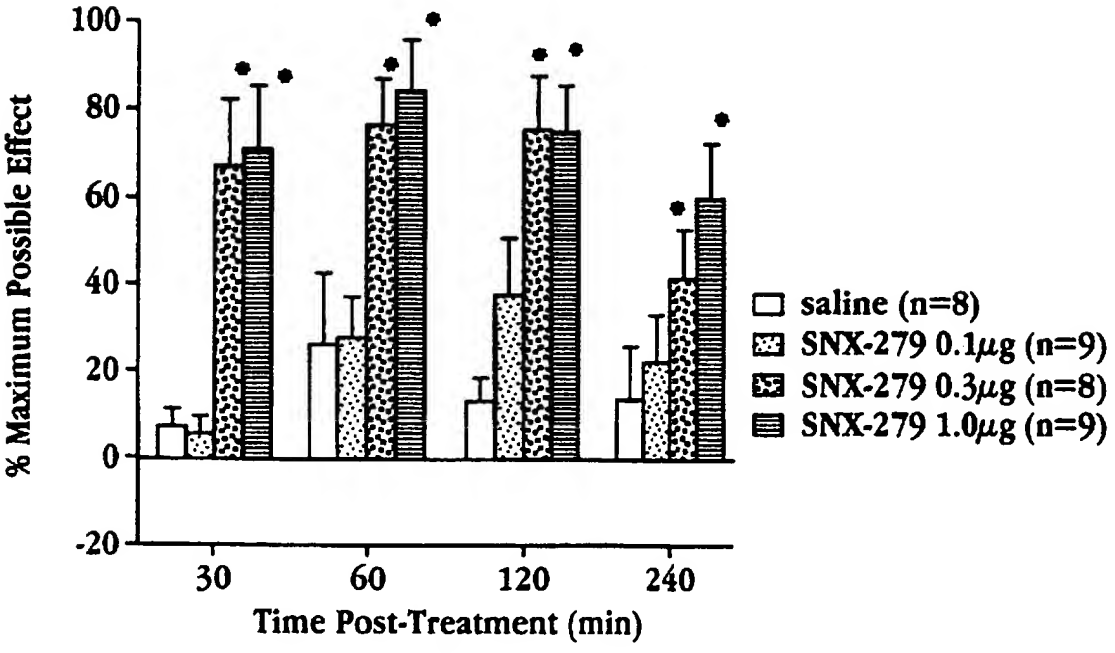
100
80
60
40
20
0
-20
% Maximum Possible Effect
30
60
120
240
Time Post-Treatment (min)
saline (n=8)
SNX-279 0.1μg (n=9)
SNX-279 0.3μg (n=8)
SNX-279 1.0μg (n=9)

Fig. 8B

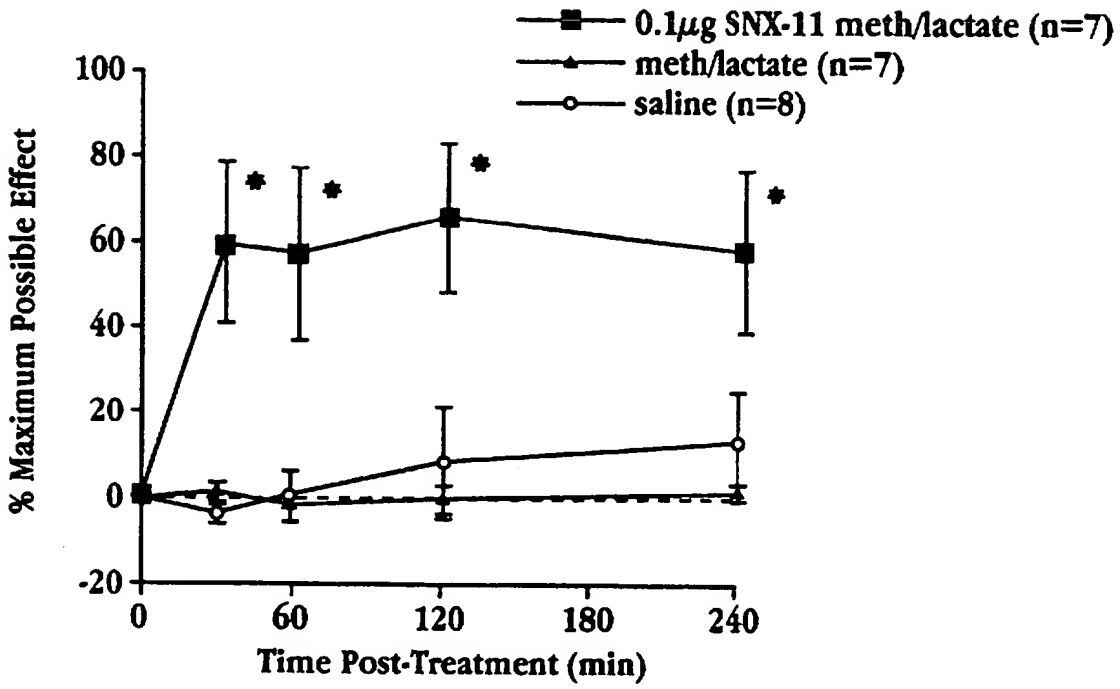
0.1μg SNX-11 meth/lactate (n=7)
meth/lactate (n=7)
saline (n=8)
% Maximum Possible Effect
100
80
60
40
20
0
-20
0
60
120
180
240
Time Post-Treatment (min)

Fig. 9

STABLE OMEGA CONOPETIDE FORMULATIONS

FIELD OF THE INVENTION

The present invention relates to methods of preventing progression of neuropathic pain and to formulations suitable for stabilizing omega conopeptide peptides used in such treatment methods.

REFERENCES

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BACKGROUND OF THE INVENTION

Chronic or intractable pain, as may occur in conditions such as bone degenerative diseases, AIDS, Reflex sympathetic dystrophy (RSD), and cancer, is a debilitating condition which is treated with a variety of analgesic agents, and often opioid compounds, such as morphine.

Neuropathic pain is a particular type of pain that has a complex and variable etiology. It is frequently a chronic condition attributable to complete or partial transection of a nerve, trauma or injury to a nerve, nerve plexus or soft tissue, or other conditions, including cancer, AIDS and idiopathic causes. Neuropathic pain is characterized by hyperalgesia (lowered pain threshold and enhanced pain perception) and by allodynia (pain from innocuous mechanical or thermal stimuli). The condition is progressive in nature. Because the hyperesthetic component of neuropathic pain does not respond to the same pharmaceutical interventions as does more generalized and acute forms of pain, development of effective longterm treatment modalities has been problematic.

Opioid compounds (opiates) such as morphine, while effective in producing analgesia for many types of pain, are generally not effective for treating the progressive stages of neuropathic pain. Moreover, these compounds are known to induce tolerance in patients, so that increased doses are required to achieve a satisfactory analgesic effect. At high doses, these compounds produce side effects, such as respiratory depression, which can be life threatening. In addition, opioids can produce physical dependence in patients. Dependence appears to be related to the dose of opioid taken and the period of time over which it is taken by the subject. For this reason, alternate therapies for the management of chronic pain are widely sought after. In addition, compounds which serve as either a replacement for or as an adjunct to opioid treatment in order to decrease the dosage of analgesic compound required, have utility in the treatment of pain, particularly pain of the chronic, intractable type.

Although calcium blocking agents, including a number of L-type calcium channel antagonists, have been tested as adjunct therapy to morphine analgesia, positive results are attributed to direct effects on calcium availability, since calcium itself is known to attenuate the analgesic effects of certain opioid compounds (Ben-Sreti, et al., 1983). EGTA, a calcium chelating agent, is effective in increasing the analgesic effects of opioids. However, results from tests of calcium antagonists as adjunct therapy to opioids have been contradictory; some L-type calcium channel antagonists have been shown to increase the analgesic effects of opioids, while others of these compounds have been shown to decrease opioid effects (Contreras, et al., 1988).

U.S. Pat. No. 5,051,403 describes the use of omega-conopeptides having defined binding/inhibitory properties in the treatment of ischemia-related neuronal damage. U.S. Pat. No. 5,364,842 demonstrates the effectiveness of omega-conopeptide compositions in certain animal models of pain. Specifically, omega-conopeptides MVIIA and TVIA and derivatives thereof having related inhibitory and binding activities were demonstrated to produce analgesia in animal models of analgesia in which morphine is the standard positive control. Co-owned application U.S. Ser. No. 08/049,794 discloses that omega conopeptides also exhibit analgesic properties in certain models of analgesia, such as neuropathic pain models of analgesia, in which morphine is not expected to produce positive results.

The present invention is based on the discovery that N-type voltage-sensitive calcium channel (VSCC) blocking compounds, including omega conopeptides, are effective to prevent progression of neuropathic conditions. Also disclosed are improved routes of administration for providing relief from neuropathic pain. In addition, the present invention discloses stabilized conopeptide formulations that are particularly useful in the treatment methods of the present invention. These stabilized compositions also find use in other applications in which prolonged administration or long-term storage of solutions containing conopeptides are required.

SUMMARY OF THE INVENTION

In one aspect, the invention includes a method of preventing progression of neuropathic pain. According to the method, the subject is given an N-type voltage-sensitive sensitive calcium channel blocking compound which is effective (a) to inhibit electrically stimulated contraction of the guinea pig ileum, and (b) to bind selectively to omega conopeptide MVIIA binding sites present in neuronal tissue. In a specific embodiment, the activities of the compound in inhibiting the guinea pig ileum and in binding to the MVIIA

binding site are within the ranges of such activities of omega-conotoxins MVIIA and TVIA. In another embodiment of the invention, the neuropathic pain is characterized by nociceptor sensitization.

In a more specific embodiment, the activity of the compound to bind selectively to the omega conopeptide MVIIA binding sites is measured by a selectivity ratio of binding at the MVIIA binding site to binding at a site 2 omega conopeptide binding site. Effective compounds will have a selectivity ratio which is within the range of selectivity ratios determined for omega conopeptides MVIIA/SNX-111, SNX-199, SNX-236, SNX-239 and TVIA/SNX-185.

In a preferred embodiment, the N-type calcium channel blocking compounds are omega-conopeptides. In another preferred embodiment, the omega conopeptide is selected from the group consisting of SEQ ID NO: 7 (TVIA/SNX-185), SEQ ID NO:1 (MVIIA/SNX-111), SEQ ID NO: 30 (SNX-236), SEQ ID NO: 2 (SNX-159), SEQ ID NO: 32 (SNX-239), SEQ ID NO: 33 (SNX-199), SEQ ID NO: 35 (SNX273), SEQ ID NO: 36 (SNX-279), and derivatives thereof.

Such peptides may preferably be administered by transdermal iontophoresis. In another preferred embodiment, the conopeptide formulation will include an anti-oxidant effective to prevent methionine oxidation.

In other preferred embodiments, the method of the invention will include administering compound by means effective to deliver compound to regions of neuropathic pain. In one preferred embodiment, the compound is administered by perineural application of compound. In another preferred embodiment, such administering is by topical application to a skin region characterized by proliferation of neurite outgrowth. Such topical administration may further include use of a transdermal patch. In another embodiment, the compound is administered by subdermal injection in a region characterized by proliferation of neurite outgrowth.

In yet another embodiment, the compound is administered by epidural injection. When practicing this method of the invention using an omega conopeptide compound, the treatment method may include means for enhancing permeation of the conopeptide through meningeal membranes. Such membrane permeation enhancing means can include, for example, liposomal encapsulation of the peptide, addition of a surfactant to the composition, or addition of an ion-pairing agent. Also encompassed by the invention is a membrane permeability enhancing means that includes administering to the subject a hypertonic dosing solution effective to disrupt meningeal barriers.

In another aspect, the invention includes a stable omega conopeptide formulation. This formulation includes, in addition to an omega conopeptide, an anti-oxidant capable of preventing methionine oxidation. In one embodiment, the anti-oxidative composition includes a carboxylic acid buffer. Generally, the buffer pH will be below about pH 6, and in a preferred embodiment, the carboxylic acid buffer is lactate buffer adjusted to a pH of about 4–4.5.

In another embodiment, the formulation includes methionine as an anti-oxidant. Generally, the formulation will be in a range below about pH 6. In a more specific embodiment, the methionine formulation buffer includes 0.9% sodium chloride acidified to a pH of about 4–4.5. The foregoing specific lactate and methionine formulations may be used alone or in combination with each other or with other anti-oxidant or stabilizing solutions known in the art. Although such anti-oxidant formulations can be used with any omega-conopeptide that is susceptible to oxidation, they are particularly useful for conopeptides SNX-111, SNX-178, SNX 207, and SNX-236.

The invention further includes, in a related embodiment a method of stabilizing an omega conopeptide solution. The method includes adding to an omega conopeptide-containing solution an anti-oxidant composition capable of preventing methionine oxidation, as described above.

In still a further aspect, the invention includes omega-conopeptide SNX-273 having the sequence: SEQ ID NO: 35. In another aspect, the invention includes omega conopeptide SNX-279 having the sequence SEQ ID NO: 36.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1A** and **1B** show primary sequences of several natural omega-conopeptides, MVIIA/SNX-111 (SEQ ID NO:01), MVIIB/SNX-159 (SEQ ID NO: 02), GVIA/SNX-124 (SEQ ID NO: 03), GVIIA/SNX-178 (SEQ ID NO: 04), RVIA/SNX-182 (SEQ ID NO: 05), SVIA/SNX-157 (SEQ ID NO: 06), TVIA/SNX-185 (SEQ ID NO: 07), SVIB/SNX-183 (SEQ ID NO: 08), and MVIIC/SNX-230 (SEQ ID NO: 29), and SNX-231 (SEQ ID NO: 21);

FIGS. **2A** and **2B** show primary sequences of analog omega-conopeptides SNX-190 (SEQ ID NO: 09), SNX-191 (SEQ ID NO: 10), SNX-193 (SEQ ID NO: 11), SNX-194 (SEQ ID NO: 12), SNX-195 (SEQ ID NO: 13), SNX-196 (SEQ ID NO: 14), SNX-197 (SEQ ID NO: 15), SNX-198 (SEQ ID NO: 16), SNX-199 (SEQ ID NO: 33), SNX-200 (SEQ ID NO: 17), SNX-201 (SEQ ID NO: 18), SNX-239 (SEQ ID NO: 32), SNX240 (SEQ ID NO: 34), SNX-202 (SEQ ID NO: 19), SNX-207 (SEQ ID NO: 20), SNX-236 (SEQ ID NO: 30), SNX-273 ([ala^{12}-SNX-111; SEQ ID NO: 35), and SNX-279 (Met(0)^{12}-SNX-111; SEQ ID NO: 36) and their relationships to SNX-111 (SEQ ID NO: 01), SNX-185 (SEQ ID NO: 07) or SNX-183 (SEQ ID NO: 08), where Nle indicates norleucine, and Met(0) indicates a sulfoxy-methionine substitution;

FIGS. **3A** and **3B** show computer-fit competitive binding curves for omega-conopeptide binding to the OCT MVIIA (SNX-111) binding site in rat brain synaptosomes;

FIGS. **4A** and **4B** show computer-fit competitive binding curves for omega-conopeptide binding to the OCT MVIIC (SNX-230) binding site in rat brain synaptosomes;

FIG. **5** shows omega-conopeptide groupings: I. MVIIA, SNX-199 (SEQ ID NO: 33), MVIIB and SNX-239 (SEQ ID NO: 32), II. TVIA, SNX-207 and SNX-236. III. RVIA, SVIA, GVIIA, SVIB, MVIIC, SNX-231;

FIGS. **6A–6D** show the effect of treatment with various omega-conopeptides on mechanical allodynia thresholds in rats with a painful peripheral neuropathy, where SNX-111 (**6A**), SNX-239 (**6B**), SNX-159 (**6C**) and SNX-230 (**6D**) were tested at the doses indicated;

FIGS. **7** (**A,B**) shows dose response curves of effects of omega-conopeptides SNX-111 (**7A**) and SNX-239 (**7B**) derived from the data illustrated in FIGS. **6A** and **6B**, respectively, in a rat model of neuropathic pain;

FIGS. **8A** and **8B** show dose-dependent blockade of mechanical allodynia by SNX-273 (**8A**) and SNX-279 (**8B**) in comparison to saline and SNX-111 at various times post treatment, where asterisks indicate statistically significant differences between treatment and saline ($p<0.05$, Student's t test); and

FIG. **9** shows analgesic efficacy of an SNX-111 formulation combining methionine in lactate buffer.

DETAILED DESCRIPTION OF THE INVENTION

I. N-type Voltage-Sensitive Calcium Channel Blocking Compounds

Voltage-gated calcium channels are present in neurons, and in cardiac, smooth, and skeletal muscle and other

excitable cells. These channels are known to be involved in membrane excitability, muscle contraction, and cellular secretion, such as in exocytotic synaptic transmission (McCleskey, et al., 1987). In neuronal cells, voltage-gated calcium channels have been classified by their electrophysiological as well as by their biochemical (binding) properties.

Calcium channels are generally classified according to their electrophysiological properties as Low-voltage-activated (LVA) or High-voltage-activated (HVA) channels. HVA channels are currently known to comprise at least three groups of channels, known as L-, N- and P-type channels (Nowycky, et al., 1985; Sher, et al., 1991). These channels have been distinguished one from another structurally and electrophysiologically as well as biochemically on the basis of their pharmacology and ligand binding properties. Thus, dihydropyridines, diphenylalkylamines and piperidines bind to the alpha, subunit of the L-type calcium channel and block a proportion of HVA calcium currents in neuronal tissue, which are termed L-type calcium currents.

N- or omega- type HVA calcium channels are distinguishable from other calcium channels by their sensitivity to omega conotoxins (omega conopeptides). Such channels are insensitive to dihydropyridine compounds, such as L-type calcium channel blockers nimodipine and nifedipine (Sher, et al., 1991; Sher and Clementi, 1991).

A. Omega-Conopeptides Omega-conopeptides are components of peptide toxins produced by marine snails of the genus Conus, and which act as calcium channel blockers (Gray, et al., 1988). About 500 species of cone snails in the Conus genus have been identified, and a variety of omega-conopeptides from several of these species have been isolated. The primary sequences of eight naturally-occurring omega-conopeptides are shown in FIG. 1, where SNX-231 is an alternative form of MVIIC/SNX-230. Conventional letter initials are used for the amino acid residues, and X represents 4-hydroxyproline, also abbreviated 4Hyp. All of the peptides shown in the figure are amidated at their C-termini.

The peptides shown in FIG. 1 are identified by names which are commonly associated with either the naturally occurring peptide (single letter followed by a Roman numeral followed by a single letter), and by a synthetic designation (SNX-plus numeral). Either or both of these designations will be used interchangeably throughout the specification. For example, the peptide whose sequence is designated MVIIA/SNX-111 will be referred to herein as OCT MVIIA, or alternatively, SNX-111, the latter to signify that the compound is synthetic in origin. Synthetic and naturally occurring peptides having the same sequence behave substantially identically in the assays and methods of treatment of the invention. The OCT MVIIA (SNX-111) and OCT GVIA (SNX-124) peptides also have the common names CmTx and CgTx, respectively. All of the omega-conopeptides have three disulfide linkages connecting cysteine residues 1 and 4, 2 and 5, and 3 and 6, as indicated for the MVIIA peptide in FIG. 2A. FIGS. 2A and 2B shows analogs or derivatives of natural OCT MVIIA, OCT TVIA, and OCT SVIB peptides which have been synthesized and tested in accordance with the invention. Standard single amino acid code letters are used in the figure; X=hydroxyproline; Nle=norleucine; Met(O)=sulfoxymethionine; NH_2 group at the C terminus indicates that the peptide is C-terminal amidated; G—OH indicates termination in an unmodified glycine residue.

B. Preparation of Omega Conopeptides

This section describes the synthesis, by solid phase methods, of several naturally occurring omega conotoxin (OCT) peptides and additional omega-conopeptides which are used in the present invention.

Omega-conopeptides, such as those shown in FIGS. 1 and 2, can be synthesized by conventional solid phase methods, such as have been described (Olivera, et al., 1984). Briefly, N-alpha-protected amino acid anhydrides are prepared in crystallized form or prepared freshly in solution and used for successive amino acid addition at the N-terminus. At each residue addition, the growing peptide (on a solid support) is acid treated to remove the N-alpha-protective group, washed several times to remove residual acid and to promote accessibility of the peptide terminus to the reaction medium. The peptide is then reacted with an activated N-protected amino acid symmetrical anhydride, and the solid support is washed. At each residue-addition step, the amino acid addition reaction may be repeated for a total of two or three separate addition reactions, to increase the percent of growing peptide molecules which are reacted. Typically, 1–2 reaction cycles are used for the first twelve residue additions, and 2–3 reaction cycles for the remaining residues.

After completing the growing peptide chains, the protected peptide resin is treated with liquid hydrofluoric acid to deblock and release the peptides from the support. For preparing an amidated peptide, the resin support used in the synthesis is selected to supply a C-terminal amide, after peptide cleavage from the resin. After removal of the hydrogen fluoride, the peptide is extracted into 1M acetic acid solution and lyophilized. The three disulfide linkages in the peptides may be formed by air oxidation in the presence of dithiothreitol (DTT) or optionally other thiol containing compounds (e.g., cysteine, glutathione), according to procedures detailed in Example 1.

The peptide can be isolated by an initial separation by gel filtration, to remove peptide dimers and higher molecular weight polymers, and also to remove undesired salts, such as guanidine hydrochloride, used in the oxidation reaction. The partially purified peptide is further purified by preparative HPLC chromatography, and the purity and identity of the peptide confirmed by amino acid composition analysis, mass spectrometry and by analytical HPLC in two different solvent systems.

C. Stable Omega-conopeptide Formulations

Dilute solutions of omega-conopeptides are generally unstable in solution, as evidenced by oxidation of methionine residues and reduction or loss of biological activity. In accordance with an important aspect of the present invention, it has been discovered that omega-conopeptides can be significantly stabilized in solution by preventing oxidation of methionine residues present in the peptide structure. In particular, SNX-111, which contains a methionine at position 12, is approximately 10-fold less potent in binding to omega-conopeptide MVIIA binding sites, when its methionine is present in the sulfoxy form.

In experiments carried out in support of the present invention, it has been found that SNX-111 oxidation can be prevented by addition of lactate buffer to the composition. More particularly buffers containing 150 mM lactate buffer, pH 4–4.5 improve stability of the compound considerably. It is known that solutions of SNX-111 in which the peptide concentration is less than about 0.1 mg/ml oxidize rapidly when dissolved in water, saline, or any of a number of buffers used in the art of peptide chemistry. It is a discovery of the present invention that solutions of SNX-111 ranging from 0.01–0.1 mg/ml are stable at 45° C. for weeks, when stabilized with lactate (150 mM, pH 4–4.5). In addition, buffers containing 50 μg/ml methionine are also effective in stabilizing SNX-111. Here, either 150 mM lactate buffer or acidified saline (pH 4–4.5) can be used to buffer the solution.

The foregoing stabilization method and formulation will find particular use in preventing oxidation of those compounds containing methionine residues. With reference to FIGS. 1 and 2, such compounds include SNX-111, SNX-178, SNX-190, SNX-191, SNX-193, SNX-194, SNX-197, SNX-198, SNX-199, SNX-200, SNX-202, SNX-207, SNX-236, SNX-237, SNX-239, SNX-240; however, it is appreciated that the formulation buffer conditions may be used with peptides that lack methionine, as well.

Formulations which incorporate the components or principles discussed above may be used in a number of pharmaceutical applications related to omega conopeptide administration. Solutions of peptides provided in vials may be stored under the acidic formulation conditions, prior to dilution into a pharmaceutical excipient suitable for parenteral administration. Solutions used for slow infusion may also be preserved in this manner. The solution may be administered directly or neutralized prior to administration, according to the particular route of administration in which the formulation is used. For example, direct intrathecal administration of approximately 10 μl of SNX-111 in lactate buffer (150 mM, pH 4–4.5) has been used in treating rats, without noticeable untoward effects. For administration to the bloodstream the acidified physiological saline formulation may prove preferable, or either preparation may be neutralized by dilution in a neutralizing physiological excipient, such as a phosphate buffered saline, just prior to administration.

D. In vitro Properties of N-type VSCC Blocking compounds

This section describes some of the in vitro properties of N-type VSCC blocking compounds, as exemplified by a specific group of omega conopeptides, namely those omega conopeptides that, like omega conopeptide MVIIA, exhibit high affinity binding to the MVIIA (site 1) binding site and relatively low affinity binding to the SVIB (site 2) omega conopeptide binding site, as discussed below.

1. Calcium-Channel Antagonist Activity. Omega conotoxins bind to a specific population of binding sites, present mainly in neuronal tissue. Dihydropyridines and other L-type channel blockers do not displace omega conotoxin binding, nor do omega conotoxins displace binding of such L-channel specific ligands to L-type calcium channels. These observations indicate that L-type calcium channel blockers and N-type calcium channel blockers act at distinct channels. Unlike L-type calcium channels, N-type or omega channels are found predominantly, although not exclusively, in nervous tissue (Sher, et al., 1991).

Inhibition (blockade) of N-type or omega HVA neuronal calcium channels can be conveniently measured in an isolated cell system, such as the mouse neuroblastoma cell line, strain N1E115 or the human neuroblastoma cell line IMR32, as described in U.S Pat. No. 5,364,842. As demonstrated therein, N-type calcium currents are blocked by omega conopeptide MVIIA, but not by dihydropyridines.

2. Specific, High Affinity Binding to OCT Receptors. Omega-conopeptides have been shown, in accordance with the invention, to bind with high affinity to specific binding site(s) in neuronal cells. In accordance with the selectivity of the compound, the binding affinity can be characterized either by the binding constant of the compound for the high-affinity MVIIA (SNX-111) binding site, also referred to as "site 1 " herein, or the binding constant of the compound for the high-affinity SVIB (SNX-183) or the MVIIC (SNX-230) binding site, also referred to as "site 2 " herein. Characteristics of these two distinct OCT binding sites are summarized below. In some cases, it is useful to characterize omega-conopeptides according to the ratio of their binding constants measured for binding to neuronal-cell MVIIA (SNX-111)-specific binding site 1 and SVIB (SNX-183)- or MVIIC (SNX-230)-specific binding site 2.

Binding to the OCT MVIIA binding site in neuronal tissue can be demonstrated in a variety of cell types and synaptosomal cell fractions. One preferred synaptosomal fraction is a mammalian brain synaptosomal membrane preparation, such as the rat brain synaptosome preparation described in U.S. Pat. No. 5,364,842. The binding constant of a compound for the MVIIA binding site is typically determined by competitive displacement of radiolabeled OCT MVIIA (SNX-111) from the synaptosomal preparation, as follows.

The binding constant (K_d) of the MVIIA (SNX-111) peptide for the synaptosomal membranes is determined by a saturation binding method in which increasing quantities of radiolabeled peptide are added to the synaptosomal membranes, and the amount of labeled material bound at each concentration is determined (Example 2A). The appropriate binding equation describing the concentration of bound ligand as a function of the total ligand in equilibrium is fitted to the data to calculate the B_{max} (the concentration of binding sites on the synaptosomes), and the K_d (which is approximately the concentration of the ligand required for half saturation of binding sites).

Using conventional Scatchard analysis, a K_d binding value of approximately 10 pM is obtained for omega conopeptide MVIIA. Similarly K_d'S were determined for binding of radiolabelled SVIB (SNX-183) and MVIIC (SNX-230) to binding sites in synaptosomal membranes.

To determine the binding constant of a test N-type VSCC blocking compound for an OCT binding site, the test compound is added, at increasing concentrations, to a membrane preparation, such as a synaptosome preparation, in the presence of a standard concentration of a radiolabeled OCT which exhibits reversible binding, such as OCT MVIIA (SNX-111). The synaptosomal material is then rapidly filtered, washed and assayed for bound radiolabel. The binding constant (K_i) of the test compound is determined from computer-fit competitive binding curves, such as shown in FIGS. 3A and 3B for MVIIA (SNX-111) peptide, to determine first the IC_{50} value of the compound, i.e., the concentration which gives 50% displacement of labeled MVIIA peptide. A K_i is determined according to standard methods from the K_d value of OCT MVIIA and the IC_{50} value of the compound, as detailed in Example 2. A relative potency value can also be calculated from this information, as illustrated. Like the K_i value, this value allows comparisons between assays performed under slightly differing conditions or at different times. While the specific value for a particular compound may vary from preparation to preparation, the rank order of binding affinities among the compounds should remain essentially unchanged. Thus the potency of a particular compound can be compared to standard compounds within a given preparation, to determine whether the test compound within a potency range considered useful in the methods of the invention, as discussed below.

Calculated IC_{50} values for a number of omega-conopeptides for binding of OCT MVIIA (SNX-111) to a rat synaptosomal preparation are given in Table 1. The compounds are arranged in order of increasing IC_{50} values.

TABLE 1

COMPETITION OF ^{125}I-MVIIA (SNX-111) BINDING BY OCT PEPTIDES

	IC_{50} (nM)
SNX-207	.007
SNX-194	.008
SNX-195	.009
MVIIA (SNX-111)	.010
SNX-190	.021
SNX-236	.030
SNX-200	.039
SNX-201	.046
SNX-202	.046
SNX-193	.070
SNX-194	.090
SNX-239	.090
MVIIC (SNX-230)	.32
MVIIB (SNX-159)	.101
GVIA (SNX-124)	.134
SNX-198	.160
SNX-191	.165
TVIA (SNX-185)	.228
SNX-196	.426
RVIA (SNX-182)	.893
SVIB (SNX-183)	1.5
GVIIA (SNX-178)	3.70
SNX-197	11.3
SVIA (SNX-157)	1460.

Similarly, IC_{50} and K_i values for compound binding to the SVIB (SNX-183) binding site can be calculated, as above, by determining the K_d of labeled OCT SVIB (SNX-183) or OCT MVIIC (SNX-230) binding to a synaptosome preparation, then using competitive displacement of the labeled compound by the test compound, to determine the IC_{50} and K_i or relative potency values of the test compound. FIGS. 4A and 4B show computer-fit competitive binding curves for several omega-conopeptides whose binding to the SVIB (SNX-183) and/or MVIIC (SNX-230) binding sites was examined. From these curves, IC_{50} values were determined as above.

Tables 2 and 3 list the relative potencies for binding of various omega-conopeptides to the site 1 and site 2 binding sites, and show the ratio of Ki or IC_{50} values determined for binding of each compound to the sites.

TABLE 2

SELECTIVITY OF CONOPEPTIDES FOR SITE 1 AND SITE 2

	Ki (nM) for competition[a] with:		Selectivity[b] for:	
Compound	[^{125}I]-SNX-111	[^{125}I]-SNX-230	site 1	site 2
SNX-111	0.002	150	75,000	1
SNX-183	0.43	6	14	1
SNX-230	0.20	0.03	1	7

[a]Ki values were derived from analysis of competitive binding performed as described in FIG. 1.
[b]Selectivity is expressed as the ratio of the Ki value determined for competition with high-affinity [^{125}I]-SNX-230 binding divided by the Ki value for competition with [^{125}I]-SNX-111 binding.

TABLE 3

SELECTIVITY OF CONOPEPTIDES FOR SITE 1 AND SITE 2

	IC_{50} (nM) for competition with:		Selectivity[a] for:	
Compound	[^{125}I]-SNX-111	[^{125}I]-SNX-230	site 1	site 2
SNX-199	0.09	5,000	56,000	1
SNX-236	0.03	1,500	50,000	1
SNX-239	0.09	10,000	111,000	1

[a]Selectivity is expressed as the ratio of the IC_{50} value determined for competition with [^{125}I]-SNX-230 binding divided by the IC_{50} value for competition with [^{125}I]-SNX-111 binding.

3. Selective Inhibition of Neurotransmitter Release. Omega-conopeptides inhibit neurotransmitter release in various regions of the nervous system. As shown below, such inhibition varies according to the neurotransmitter, the omega-conopeptide, and the region studied. Neurotransmitters which can be measured, in accordance with various aspects of the invention, include, but are not limited to dopamine, norepinephrine, acetylcholine, GABA, glutamate, and a number of peptide neurotransmitters, such as calcitonin gene-related peptide (McGeer, et al., 1987).

Quantitation of release and inhibition thereof is determined by sensitive detection methods, also known in the art, including direct detection of release of endogenous stores by HPLC or specific radioimmunoassay (RIA), and detection of release of pre-loaded, labeled compound. Alternatively, or in addition, detection of release may be achieved using a number of indirect assays, exemplified by the electrophysiological studies described above, in which whole tissue response to electrical or chemical stimulation is measured.

Inhibition of release of the neurotransmitter norepinephrine from neuronal cells can be assayed in a number of systems known in the art, and, particularly, in mammalian brain hippocampal slices by standard methods, such as detailed in U.S. Pat. No. 5,364,842. According to the data shown therein, SNX-111 inhibits release of norepinephrine with high potency (IC_{50}≈1 nM) but only partially (approx. 60%). SNX-183 is much less potent (IC_{50}≈180 nM) but the inhibition is substantially 100%. SNX-230 also inhibits release completely, but in a biphasic manner, inhibiting approximately 50% with high potency (IC_{50}=0.02 nM) and 50% with much lower potency (IC_{50}=65 nM). These results suggest that such norepinephrine release is mediated by at least two distinct subtypes of presynaptic calcium channels, one of which corresponds to the site 1 receptor identified by high affinity for SNX-111 and the other to the site 2 receptor recognized preferentially by SNX-230.

Further means of measuring inhibition of neuronal transmitter release are isolated tissue assays, such as atrial strip, aorta, vas deferens and guinea pig ileum assays, in which the response to a stimulus, usually an electrical stimulus, is correlated to the amount of neurotransmitter released from neurons innervating the tissue (Kenakin, 1987). In the guinea pig ileum, inhibition of electrically stimulated contractions is correlated with inhibition of acetylcholine release, as demonstrated by the ability of cholinergic agonists to overcome such inhibition. Example 3 describes the preparation and assay in detail. Table 4 shows the IC_{50} values for various omega-conopeptides on contraction of guinea pig ileum in response to electrical stimulation.

TABLE 4

EFFECTS OF CONOPEPTIDES ON ELECTRICALLY STIMULATED CONTRACTION OF GUINEA PIG ILEUM

Compound	ID_{50} (nM)
SNX-111	13
SNX-185	29
SNX-183	91
SNX-157	>100

II. Treatment of Neuropathic Pain

U.S. Pat. No. 5,364,842 describes analgesic properties of selected omega-conopeptides. This discovery was extended to include treatment of neuropathic pain as disclosed in co-owned U.S. patent application Ser. No. 08/049,794, filed Apr. 15, 1993, and incorporated herein by reference. It is the discovery of the present invention that progression of neuropathic pain can be retarded in a subject exhibiting early stage symptoms of neuropathic pain, particularly by providing localized delivery of N-type VSCC blocking compounds to the neuropathic site.

A. Prevention of Progression of Neuropathic Pain

In general, while brain pathways governing the perception of pain are still incompletely understood, sensory afferent synaptic connections to the spinal cord, termed "nociceptive pathways" have been documented in some detail. In the first leg of such pathways, C- and A-fibers which project from peripheral sites to the spinal cord carry nociceptive signals. Polysynaptic junctions in the dorsal horn of the spinal cord are involved in nociceptive processing and in the relay of sensations of pain to various regions of the brain. Analgesia, or the reduction of pain perception, can be effected directly by decreasing transmission along such nociceptive pathways. Modulation of nociception is achieved by neural pathways descending from the cortex and hypothalamus to the mesencephalic central grey region, medullary reticular formation, and ultimately, the dorsal horn of the spinal cord. Analgesic opiates are thought to act by mimicking the effects of endorphin or enkephalin peptide-containing neurons, which synapse presynaptically at the C- or A-fiber terminal and which, when they fire, inhibit release of neurotransmitters, including substance P, excitatory amino acids and calcitonin gene-related peptide (CGRP). Descending pathways from the brain are also inhibitory on C- and A-fiber firing.

While neuropathic pain is known to have a number of underlying etiologies, it is characterized by a distinct set of symptoms. As described in greater detail below, these can include enhanced sensitivity to innocuous thermal-mechanical stimuli, abnormal sensitivity to noxious stimuli, tenderness, and spontaneous burning pain. Neuropathic pain is also progressive in nature, in that it generally worsens over time. Known treatment methods treat the symptoms without necessarily lessening the underlying pathology.

Although the present invention should not be limited by a particular theory of underlying mechanism, it has been observed that chronic neuropathic pain in humans is accompanied by changes in excitability of primary nociceptive afferents. This phenomenon is known as "nociceptor sensitization" and is characterized by increased excitability of the afferents to normally subthreshold stimuli. It is the discovery of the present invention that treatment with omega conopeptides and, more generally, N-type VSCC blockers, inhibit this phenomenon.

In accordance with the present invention, omega-conopeptides useful in treating neuropathic pain are also effective in preventing its progression. Such "neuropatholytic" omega-conopeptides may be distinguished and selected by their abilities (a) to inhibit voltage-gated calcium channels selectively in neuronal tissue, as evidenced by the peptide's ability to inhibit electrically stimulated contraction of the guinea pig ileum, and (b) to bind to omega conopeptide MVIIA binding sites present in neuronal tissue. Such binding to omega conopeptide MVIIA binding sites (site 1, as described herein) is selective, as evidenced by relatively high binding affinity at such sites, as compared to binding at an omega conopeptide site 2 (described herein as a high affinity binding site for SNX-230 or SNX-183). Such selectivity can be measured by the selectivity ratio illustrated in Tables 2 and 3, above.

Moreover, in accordance with the invention, it has been found that neuropatholytic omega-conopeptides are effective as analgesic agents both in traditional opiate-sensitive models of nociceptive pain, such as the Rat Tail-Flick test or the rat hind-paw formalin test, as well as in opiate-resistant models of pain, such as chronic constriction injury models of neuropathic pain.

B. Treatment Modes for Preventing Progression of Neuropathic Pain

While the present invention is not intended to be limited by adherence to any particular theory of underlying mechanism, one theory which is consistent with the invention is that acute nociception leads to alteration of signal transduction mechanisms in pain pathways. Under this theory, interference with such signal transduction by blockade of N-type VSCC's prevents this alteration.

It is a discovery of the present invention that N-type calcium channel blocking omega-conopeptides are effective to prevent development of or progression of neuropathic pain, when administered perineurally to affected skin regions characterized by proliferation of neurite outgrowth subsequent to nerve damage.

Perineural administration can be by topical means, either directly or with the use of a transdermal applicator. Alternatively, perineural administration may be effected by subdermal injection. The resulting blockade of calcium channels reduces the heightened sensation produced by transmission through the neurite proliferation. Perineural delivery may also be effected by forming a cuff around the damaged nerve, preferably of a biodegradable matrix which includes a therapeutic N-type calcium channel blocking compound. Alternatively or in addition, the therapeutic compound can be placed in close proximity to the damaged nerve by conjugating the compound to or coating the compound on a nerve splint designed for repairing damaged nerves. Examples of such nerve splints are provided by U.S. Pat. Nos. 4,534,349 and 4,920,962.

Perineural delivery may also be effected by incorporating N-type calcium channel blocking compounds into suture materials, and using these materials to suture damaged tissues. This method is particularly useful for delivery of compound in areas where it is desirable to provide for inhibition of progression of neuropathy concomitant to tissue damage. U.S. Pat. No. 5,308,889 describes a collagen-based suture material that may be suitable for use for therapeutic delivery of N-type calcium channel blocking compounds.

One particularly useful means for delivering compound to perineural sites is transdermal delivery. This form of delivery can be effected according to methods known in the art. Generally, transdermal delivery involves the use of a transdermal "patch" which allows for slow delivery of compound to a selected skin region. Although such patches are gener-

ally used to provide systemic delivery of compound. in the context of the present invention. such site-directed delivery can be expected to provide increased concentration of compound in selected regions of neurite proliferation. Examples of transdermal patch delivery systems are provided by U.S. Pat. No. 4.655.766 (fluid-imbibing osmotically driven system). and U.S. Pat. No. 5.004.610 (rate controlled transdermal delivery system).

For transdermal delivery of peptides. such as omega-conopeptides described herein. transdermal delivery may preferably be carried out using iontophoretic methods. such as described in U.S. Pat. No. 5.032.109 (electrolytic transdermal delivery system). and in U.S. Pat. No. 5.314.502 (electrically powered iontophoretic delivery device).

For transdermal delivery. it may be desirable to include permeation enhancing substances. such as fat soluble substances (e.g., aliphatic carboxylic acids. aliphatic alcohols). or water soluble substances (e.g., alkane polyols such as ethylene glycol. 1.3-propanediol. glycerol. propylene glycol. and the like). In addition. as described in U.S. Pat. No. 5.362.497. a "super water-absorbent resin" may be added to transdermal formulations to further enhance transdermal delivery. Examples of such resins include. but are not limited to. polyacrylates. saponified vinyl acetate-acrylic acid ester copolymers. cross-linked polyvinyl alcohol-maleic anhydride copolymers. saponified polyacrylonitrile graft polymers. starch acrylic acid graft polymers. and the like. Such formulations may be provided as occluded dressings to the region of interest. or may be provided in one or more of the transdermal patch configurations described above.

For delayed release. the N-type calcium channel blocking compound may be included in a pharmaceutical composition for formulated for slow release. such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems according to methods known in the art.

For continuous release of peptides. the peptide may be covalently conjugated to a water soluble polymer. such as a polylactide or biodegradable hydrogel derived from an amphipathic block copolymer. as described in U.S. Pat. No. 5.320.840. Collagen-based matrix implants. such as described in U.S. Pat. No. 5.024.841. are also useful for sustained delivery of peptide therapeutics. Also useful. particularly for subdermal slow-release delivery to perineural regions. is a composition that includes a biodegradable polymer that is self-curing and that forms an implant in situ. after delivery in liquid form. Such a composition is described. for example in U.S. Pat. No. 5.278.202.

The method of the invention also includes local administration of compound to those regions of the spinal cord. such as to dorsal horn regions at affected vertebral levels. where polysynaptic relay of pain sensation occurs. This type of local application can be effected by intrathecal administration. as described in above-referenced co-pending application U.S. Ser. No. 08/049.794. Intrathecal administration delivers compound directly to the sub-arachnoid space containing cerebral spinal fluid (CSF). While effective. this method requires precise technical expertise to ensure delivery to the correct spot.

It is appreciated that delivery to spinal cord regions can also be by epidural injection to a region of the spinal cord exterior to the arachnoid membrane. It is further appreciated that when the N-type voltage-sensitive calcium channel (VSCC) blocking compound is an omega conopeptide. it may be advantageous to add to the conopeptide composition means for enhancing permeation of the conopeptide through meningeal membranes. Such means are known in the art and include liposomal encapsulation. or addition of a surfactant or an ion-pairing agent to the peptide composition.

Alternatively. or in addition. increased arachnoid membrane permeation can be effected by administering a hypertonic dosing solution effective to increase permeability of meningeal barriers.

N-type calcium channel blocking compounds can also be administered by slow infusion. This method is particularly useful. when administration is via the intrathecal or epidural routes mentioned above. Known in the art are a number of implantable or body-mountable pumps useful in delivering compound at a regulated rate. One such pump described in U.S. Pat. No. 4.619.652 is a body-mountable pump that can be used to deliver compound at a tonic flow rate or at periodic pulses. An injection site directly beneath the pump is provided to deliver compound to the area of need. for example. to the perineural region.

In other treatment methods. N-type calcium channel blocking compounds may be given orally or by nasal insufflation. according to methods known in the art. For administration of peptides. it may be desirable to incorporate such peptides into microcapsules suitable for oral or nasal delivery. according to methods known in the art.

Efficacy of the foregoing methods of treatment are conveniently measured in the allodynia model described in Example 4 herein. In this model of peripheral neuropathy. it has been observed that neuropathic pain progressively increases over days 1–7 after the nerve insult. with a plateau of pain response thereafter. To measure ability of a compound to prevent such progression. compound is given just before. during or after nerve insult. and threshold pain responses are measured on days 1–7 thereafter. Prevention of progression of neuropathic pain is observed when there is a heightened threshold to pain stimulus during days 1–7 or where there is a prolongation of time to plateau. as compared to control animals. Efficacious dosages and formulations determined in this model are extrapolated to equivalent large animal and human dosages. according to methods known in the art.

C. Therapeutic Indications

As stated above. neuropathic pain may result from a number of separate etiologies. Generally. progression of such pain may be treated according to any of the methods described herein. However. in many cases it will be preferable to treat the pain in a manner that addresses its specific source. For example. when the pain is traceable to injury of a particular nerve fiber. it may be appropriate to treat such pain either by perineural application of compound to the affected nerve or by dermal application of compound to the affected region.

While a discussion of specific formulations and modes of delivery can be found in foregoing Section II.B. and in Section II.F. below. this section sets forth some exemplary indications for which treatment according to the methods of the present invention may have particular therapeutic utility. The indications described below are by no means exhaustive. but are presented to illustrate some of the various therapeutic situations in which neuropatholytic N-type calcium channel blocking compounds can be used.

1. Ophthalmic indications. The eye is a heavily innervated organ. The cornea in particular is heavily innervated with C-fibers. containing an estimated 3–4000 fiber endings per mm^2 compared to an estimated 3–600 fiber endings per mm^2 of skin. Injury of the nerve fibers can lead to neuropathic pain of ophthalmic origin. In accordance with the present invention. the eye may be treated with N-channel blocking compounds and particularly with omegaconopeptides. to

prevent progression of neuropathy. Application of compound may be achieved by topical administration to the eye, or, in more severe cases, by means of suprachoroidal administration. Such administration may be conveniently achieved by providing a suprachoroidal implant which includes, for example, omega conopeptide SNX-111. U.S. Pat. No. 5,164,188 describes a biodegradable implant that is suitable for chronic and controlled administration of compound to the suprachoroidal space. Chronic, implanted therapeutics are also indicated after ophthalmic surgery, such as after surgery for detached retina or macular holes, where nerve damage may result.

2. Dental indications. Delivery of N-channel blocking compounds to regions of dental repair, such as endodontic repair concomitant to a root canal procedure, may be desirable as a means of preventing progression of dental neuropathy. Here, the therapeutic compound may be included in or added to one or more of the polymer based materials or resins inserted into the root canal after removing the pulp from the region, in accordance with standard techniques known in the art.

3. Burn injury. Burn injuries are characterized by primary hyperalgesia to thermal and mechanical stimuli. In accordance with the principles discussed above, treatment of burned regions with N-type calcium channel blocking compounds may reduce progression of the hyperalgesic response by interfering with signal transduction mechanisms of nociceptor sensory receptors. In this embodiment of the invention, the therapeutic compound can be applied directly to the affected regions, or can be applied in a formulation that includes a protective biopolymer matrix, such as an artificial skin matrix.

4. Reflex sympathetic dystrophy (RSD). RSD is thought to be due to abnormalities in the peripheral nervous system, and more particularly, to sensitization of cutaneous somatosensory afferents. Sympathetic outflow is thought to activate foci of ectopic neural hyperexcitability. Treatment of this condition to prevent its progression may be effected by any of the various dermal (topical) or subdermal routes of delivery discussed above. Perineural delivery may also be indicated for this condition.

5. Post-herpetic neuralgia. Post-herpetic neuralgia is characterized, in its acute phase, by intraneural inflammation which can cause damage to primary afferent axons. This damage may result in abnormal sensitivity to cutaneous stimuli. In general, the mode of treatment to prevent progression of abnormal sensitivity will depend on the location in the body of the affected nerve(s). Perineural or topical delivery of therapeutic N-type calcium channel blocking compound is indicated in this condition.

6. Diabetic neuropathy. Neuropathy of primary afferent axons in long nerves is found in diabetic patients. This results in the dying-back and attempted regeneration of distal tips of primary afferent axons of these nerves. Nociceptor sensitization may ensue. Such sensitization and its progression may be treated according to one or more of the treatment methods described herein. In particular, perineural or topical application of therapeutic compound will be indicated, in accord with the location of the affected nerve and nerve beds.

7. Arthritis. Arthritis is characterized by enhanced sensation of pain via articular afferents. N-type calcium channel blocking compounds find utility in treatment of such pain according to the principles set forth in the present invention. Generally, in treating articular afferents, therapeutic compound will be administered perineurally, in the vicinity of the affected joint.

D. Omega-Conopeptides

In accordance with this invention, neuropatholytic omega-conopeptides conform to certain physical and chemical constraints, as described below. Generally, omega-conopeptides useful in the treatment methods are those which are 25–35 amino acids in length and which have three disulfide bonds at specified positions along their length.

Based on a sequence homology analysis of the peptides whose full sequences are known (FIG. 1), the naturally occurring active omega-conopeptides were grouped into distinct groups I and II, each with internal homologies distinct to that group, as can be appreciated from FIG. 5. Group I includes active omega-conopeptides MVIIA (SNX-111), MVIIB (SNX-159) and SNX-239, which possess binding constants to the MVIIA site within the range of compounds showing activity in treating pain. Group II includes TVIA (SNX-185), SNX-207 and SNX-236, which also possess binding constants in the range of compounds for analgesia. A third group includes inactive peptides SNX-231, and SVIA (SNX-157) and omega-conopeptides whose binding activities for the MVIIA site on neuronal membranes and/or activity in norepinephrine inhibition are outside the range of active compounds.

The three groups of omega-conopeptides are arranged in FIG. 5 with their six Cys residues aligned, which places these residues at positions 1, 8, 15, 16, 20, and 28. To make this alignment, gaps were introduced at the positions shown in the three groups. In the analysis below, these gaps retain the assigned number shown in FIG. 5, even though they represent amino acid deletions in the respective groups of active omega-conopeptides.

Sequence variation in the peptides, based on primary structure alone, was analyzed by adopting the following constraints:

1. The peptides in both active groups (I and II) include the Cys residues at position 1, 8, 15, 16, 20, and 28. Other Cys residues could be substituted at the positions indicated below only if they are selectively protected during oxidation of the peptide to form the three disulfide linkages.

2. The peptides in the active groups include three disulfide linkages connecting the Cys residues at positions 1 and 16, 8 and 20, and 15 and 28. As described above, the disulfide bridges are formed by air oxidation of the full sequence peptide in the presence of DTT. The ability of the peptide to form the three desired disulfide linkages would therefore require that the peptide, prior to disulfide bridging, be able to adopt a conformation which allows the three selected linkages, with or without the Cys protecting-group strategy discussed above. This constraint would thus exclude amino acid variations which prevent or otherwise hinder the formation of the three selected bridges.

Constraints 1 and 2 preserve the basic conformation of the omega-conopeptides imposed by the three disulfide bridges.

3. Within Group I, the amino acid variations which occur at the six non-conserved residues are allowed, including peptides in which the carboxy terminus is amidated or has a free acid form. That is, the first group compound derivatives include the peptide structures having the form: SEQ ID NO: 22-X_1-SEQ ID NO: 23-X_2-X_3-X_4-X_5-SEQ ID NO: 24-X_6-SEQ ID NO: 25-X_7-SEQ ID NO: 26-t, where X_1=K or S; X_2=S or H; X_3=R, L, or A; X_4=L or T; X_5=M or S; X_6=N or a deletion; SEQ ID NO 25 is R; X_7=S or deletion, and t=a carboxy or amidated carboxyterminal group, and where SEQ ID NO: 22 is C K G K G A; SEQ ID NO: 23 is C; SEQ ID NO: 24 is Y D C C T G S C; and SEQ ID NO: 26 is G K C.

4. Within Group II, the amino acid variations which occur at the eight non-conserved residues are allowed, including

peptides in which the carboxy terminus is amidated or has a free acid form. Thus, the second group compound derivatives include the peptide structures having the form: SEQ ID NO: 27-$X_1X_2X_3$-SEQ ID NO: 28-X_4-SEQ ID NO: 31-t, where X_1=X or R; X_2=T or L; X_3=S or M, X_4=X or P; and t=a carboxy or amidated carboxyterminal group, and where SEQ ID NO: 27 is C L S X G S S C S; SEQ ID NO: 28 is Y N C C R S C N; and SEQ ID NO: 31 is Y S R K C R.

5. Considering both active groups together, amino acid positions which are conserved in all active species are preserved. Thus, for example, the Cys residues, the 5-position glycine, the 13-position tyrosine, the 19-position serine, and the 26-position lysine are all preserved. Preferred OCT analogs or derivatives may be selected by comparing, for purposes of inter-sequence conservation and substitution, those sequences known to be active. For example, in the case of the treatment of pain, omega-conopeptides MVIIA (SNX-111), SNX-239, SNX-199, TVIA (SNX-185) and SNX-236 are known active compounds. Active derivatives are those peptides having, in addition to the conserved cysteine residues described above, a conserved glycine residue at position 5, conserved serine residues at positions 9, 19, and 24, and a conserved lysine residue at position 26. Inter-sequence substitution of variable residues is then preferable in the formation of active analogs. For example, analog position 2 may be occupied by a lysine or a leucine residue, and position 6 may be occupied by an alanine or a serine residue.

6. Considering both active groups together, there are amino acid positions which are likely to be variable within the range of active species. For example, the position 2 amino acid may be lysine or leucine, the position-3 amino acid may be glycine or serine, and the position 4 amino acid, hydroxyproline or arginine. In addition, if the two or more amino acids at a variant position are in a common substitution class, substitution within that class may be favorable. Standard substitution classes are the six classes based on common side chain properties and highest frequency of substitution in homologous proteins in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix (Dayhoff). These classes are Class I: Cys; Class II: Ser, Thr, Pro, Hyp, Ala, and Gly, representing small aliphatic side chains and OH-group side chains; Class III: Asn, Asp, Glu, and Gln, representing neutral and negatively charged side chains capable of forming hydrogen bonds; Class IV: His, Arg, and Lys, representing basic polar side chains; Class V: Ile, Val, and Leu, representing branched aliphatic side chains, and Met; and Class VI: Phe, Tyr, and Trp, representing aromatic side chains. In addition, each group may include related amino acid analogs, such as ornithine, homoarginine, N-methyl lysine, dimethyl lysine, or trimethyl-lysine in class IV, and a halogenated tyrosine in Group VI. Further, the classes may include both L and D stereoisomers, although L-amino acids are preferred for substitutions.

7. Considering the known inactive species, substitutions to amino acids which are present in inactive species, but not active ones, at any selected residue position, are not favored to preserve activity in the active compounds. Thus, for example, although a 3-position serine is present in both active and inactive compounds, 4-position serine or threonine is present in inactive species only, and either substitution is thus disfavored.

The above amino acid selection rules 6–7 are intended as a guide for allowed amino acid substitutions within active omega-conopeptides. Once an amino acid substitution or modification is made, the peptide is further screened for the requisite calcium channel antagonist activity, and the requisite activities for inhibition of neurotransmitter release and binding to the appropriate OCT binding site of neuronal membranes, as described above.

Several of the amino acid substitutions or modifications to the omega-conopeptide illustrate the principles outlined above.

Omega-conopeptides which are selected on the basis of these criteria, discussed in detail below, are tested for ability to produce analgesia in a standard test of analgesia, such as the Rat Tail-Flick test, wherein analgesia is measured by a prolongation of reaction time to a noxious radiant heat stimulus.

E. In vitro Properties of Neuropatholytic Omega-Conopeptides

1. Calcium Channel Blocking Activity.

Calcium channel blocking activity was measured electrophysiologically in neuronal (N1E-115 or IMR-32) cell lines, as described in Section II. Omega-conopeptides having calcium channel blocking activity are those which block calcium currents in such cell lines with potencies in the range observed for omega-conopeptides MVIIA and GVIA in N1E-115 cells.

2. High Affinity Binding to OCT Binding Sites. Methods for determination of binding affinity to OCT binding sites are detailed in Examples 2–3, below.

Compounds were tested for their ability to displace binding of SNX-111, SNX-183, or SNX-230 from their respective binding sites (site 1 and site 2, as described above). In displacing SNX-111, it was found that compounds having analgesic activity, such as OCT MVIIA (SNX-111), SNX-239, SNX-236, SNX-199 SNX-159 and TVIA (SNX-185), have IC_{50} values between about 15 and 300 pM, and K; values between about 1 and 100 pM. In contrast inactive compound SNX-183 had an IC_{50} of greater than 1000 pM for binding at the MVIIA site.

From the foregoing, it is seen that active compounds in accordance with the invention are characterized by a high binding affinity for MVIIA binding site 1. The binding affinity for these sites may be characterized as follows. In the first approach, the binding affinity of the compound for binding site 1, as estimated by IC_{50} in displacing MVIIA from the site, is compared directly with those of selected high affinity active compounds, such as SNX-111 and SNX-185. An active compound is one whose binding affinity is at least as high as and preferably within the range of binding affinities measured for such high affinity OCT's. Secondly, the binding affinity of the test compound can be characterized by binding to binding site 2, as estimated by IC_{50} in displacing MVIIC (SNX-230) or SVIB (SNX-183) from the site. Thirdly, the binding affinity of the compound can be characterized by the ratio of binding constants or relative affinities of the compound for site 1 and site 2, as just described. Here an active compound is one whose binding ratio is within the range for the selected active peptides, such as MVIIA (SNX-111) and TVIA (SNX-185); i.e., the binding ratio is substantially within the range of the ratio observed for the omega-conopeptides MVIIA and TVIA.

A number of omega-conopeptide compounds which were tested gave IC_{50} and K_i values lower than or within the ranges of those of omega-conopeptides MVIIA (SNX-111) and TVIA (SNX-185) for binding at the SNX-111 site, as shown in Table 1, and these compounds should thus be considered candidates as analgesic compounds. In addition active compounds have relatively high selectivities of binding, equivalent or greater than those of MVIIA and TVIA, as shown in Tables 2 and 3. However, some of these

compounds, may not fulfill additional criteria for analgesic compounds of the invention, as described herein.

3. Inhibition of neurotransmitter release. Another requisite property of analgesic OCT compounds, in accordance with the invention, is their ability to specifically inhibit depolarization-evoked and calcium-dependent neurotransmitter release from neurons. For example, it is shown here that analgesic omega-conopeptides inhibit of electrically stimulated release of acetylcholine at the myenteric plexus of the guinea pig ileum (Example 3). This inhibition is associated anti-nociceptive activity, as seen in Table 4. Omega-conopeptides having analgesic activity have IC_{50}'s in the range of those values observed for active omega-conopeptides MVIIA (SNX-111) and TVIA (SNX-185), or less than approximately 50 nM, as observed in this assay.

4. In vivo Measurements of Neuropathic Analgesia.

Relief of neuropathic pain is conveniently measured in one or more of a number of animal models known in the art, in which an animal's response to a given pain stimulus is measured following experimental production of neuropathy. Inhibition of progression of the neuropathic condition can also be measured in this model, when measurements are taken in individual animals over time following the experimental neuropathic insult. While normal progression of the condition will be expected to result in animals that are increasingly sensitive to a given pain stimulus over time, inhibition of this progression will be observed as a leveling or diminishing of response to pain stimulus with time.

One demonstrated model of neuropathic pain resembles the human condition termed causalgia or reflex sympathetic dystrophy (RSD) secondary to injury of a peripheral nerve. This condition is characterized by hyperesthesia (enhanced sensitivity to a natural stimulus), hyperalgesia (abnormal sensitivity to pain), allodynia (widespread tenderness, characterized by hypersensitivity to tactile stimuli), and spontaneous burning pain. In humans, neuropathic pain tends to be chronic and may be debilitating. This type of pain is generally considered to be non-responsive or only partially responsive to conventional opioid analgesic regiments (Jadad, et al., 1992). In accordance with the invention, neuropatholytic N-type VSCC blocking compounds are effective in providing relief of neuropathic pain, as described below.

Experiments carried out in support of the present invention were performed in a rat model of peripheral neuropathy detailed in Example 4. Briefly, in the model used, rats are subjected to a surgical procedure, described by Kim and Chung (1992) and Bennett and Xie (1988) designed to reproducibly injure peripheral nerves (spinal nerves L5 and L6). These rats develop a hyperesthetic state, which can be measured, using one or more paradigms known in the art. Here, mechanical allodynia was measured by stimulation of neuropathic rat hindlimb using wire hairs having graded degrees of stiffness. Analgesic compounds reverse the heightened sensitivity such animals exhibit to the stimulus.

FIG. 6 shows results in the allodynia test of animals treated with SNX-111 (6A), SNX-239 (6B), SNX-159 (6C) and SNX-230. Data are expressed as percent maximum effect, where the maximum effect indicates a complete reversal of surgically induced allodynia, or relative insensitivity to stimulus (maximum equals 15 gram hair stimulus). A baseline of zero indicates a mean sensitivity to a wire hair graded at less than 3 grams. As shown in FIG. 6A, treatment of rats (n=6/treatment) with 1 or 3 μg SNX-111 resulted in elevation of threshold response. Peak elevation of response due to drug treatment (reversal of allodynia) was observed by 30–60 minutes, and effects lasted in excess of 60 minutes. SNX-239 showed significant analgesic effects at a dose as low as 0.33 μg, and evoked a prolonged analgesic response of at least 2 hours, as indicated. SNX-159 was also effective against neuropathic pain in this test at submicromolar doses (FIG. 6C), while SNX-230was ineffective at such doses (FIG. 6D).

FIGS. 7A and 7B show dose response curves derived from the data shown in FIGS. 6A and 6B. These results indicate that analgesic omega conotoxin peptides, exemplified by SNX-111, are capable of reversing the hyperesthetic effects induced by nerve damage.

F. Dosages and Formulations

From the foregoing, it can be appreciated that treatment with N-channel blocking compounds and, more particularly, omega conopeptides having binding and inhibitory activities within the range of activities defined by omega conopeptides MVIIA (SNX-111) and TVIA (SNX-185) are useful in preventing progression of neuropathic pain. Generally, dosages and routes of administration of the compounds will be determined according to the site of the pain and the size of the subject, according to standard pharmaceutical practices. Intrathecal administration, either as a bolus dosage and as a constant infusion, can be used for treatment and prevention of progression of neuropathic pain. In preferred embodiments, dosages equivalent to at least 0.1–3 μg intrathecal SNX-111 in rats are effective against peripheral neuropathy of the hindlimb. It is also appreciated that compound can be administered perineurally, for example by topical or subdermal application to cutaneous regions having affected nerve endings, according to methods known in the art. In addition, administration may by epidural means, as discussed below.

Stabilized formulations, as described in Section I.B above, are useful in storing and administering therapeutic omega conopeptides according to the methods described herein. While it may be desirable to neutralize the solutions prior to administration, formulations utilizing lactate or acidified saline as excipient may also be administered directly via acute intrathecal bolus injection or by other routes for which acidified excipients are appropriately used. Neutralization, if required, can be accomplished by dilution into a pharmaceutically acceptable neutralizing excipient buffer, just prior to injection into the subject. FIG. 9 shows the results of experiments demonstrating that SNX-111 administered as a methionine-lactate formulation was as effective as SNX-111 alone in reducing neuropathic pain.

For some applications, it may be desirable to include in the omega conopeptide composition or treatment regimen means for enhancing permeation of the conopeptide through meningeal tissues which may surround the damaged or target nerve. Means for enhancing transport of compound are known in the art and may include encapsulating the conopeptide in liposomal membranes, addition of a surfactant to the composition, addition of an ion-pairing agent, and the like. Alternatively, or in addition, transmeningeal transport may be facilitated by administering to the subject a hypertonic dosing solution effective to disrupt meningeal barriers, according to methods well known in the art. Alternatively, trans meningeal or transcutaneous transport may be facilitated by modifying the primary sequence of omega-conopeptide, for example by substituting neutral amino acid sidechains or hydrophobic moieties for cationic residues.

The following examples are intended to illustrate various characteristics of the method of the invention, but are in no way intended to limit the scope of the invention.

EXAMPLE 1

Preparation of OCT Peptides

Abbreviations used in this example are BOC, tertiary butoxycarbonyl; DCM, dichloromethane; TFA, trifluoroace-

tic acid; IPM, N-isopropylmorpholine; BOC-AA-OH, BOC amino acid; DIEA, diisopropylethylamine; 2-ClZ, chlorobenzyloxycarbonyl; tosyl, p-toluenesulfonyl; DMF, N,N-dimethylformamide; TFE, trifluoroethanol; SA, symmetrical anhydride of BOC-AA-OH; DCCI, N,N-dicyclohexylcarbodiimide; E, ethyl ether; P, petroleum ether.

Commercially available benzhydrylamine-resin hydrochloride, Lot No. B30101, was obtained from commercial sources (Beckman Instruments Inc., Palo Alto, Calif. Advanced ChemTech). With this resin, cleavage of a peptide formed on the resin, under the conditions described below, produces a peptide which is amidated at its carboxy end.

A. Preparing Protected Amino Acid Anhydrides Each BOC-AA-OH (2.4 mmol) was dissolved in 5 ml CH_2Cl_2 and cooled to 0 C. The volume of DCM used for BOC-Leu-OH (dried in vacuo) was 12 ml, and the BOC-Leu-OH solution was not cooled. 2 ml 0.6 M DCCI in DCM was added and the mixture stirred at 0 C. for 15 min. For BOC-Leu-OH, the mixture was also cooled after this addition. Precipitation of N,N-dicyclohexylurea was completed by storage at −20° C. for 1.5 hour, after which the precipitate was filtered and washed with ethyl ether (5 ml). The filtrate was evaporated to remove solvents and the product was crystallized in the solvent system given in the Table below. Residual amounts of DCM can affect the exact conditions for crystallization. Recrystallization was performed by dissolving in DCM, evaporating most of the solvent, and recrystallizing from the appropriate solvent.

TABLE OF AMINO ACID SOLVENTS

Amino Acid	Solvent
Ala	DCM:E:P
Asp (Benzyl)	E:P
Gly	E:P
Leu	P
Lys (2-ClZ)	E:P
Met	E:P
Ser (Benzyl)	E:P
Thr (Benzyl)	E:P
Tyr (2-BrZ)	DCM:P

B. Preparation of MVIIA

Synthesis of MVIIA peptide was performed on 0.58 g benzhydrilamine resin (0.40 mmol) in a Beckman Model 990 Peptide Synthesizer by a solid-phase method based on the primary structure shown in FIG. 1A.

A double coupling protocol was used for the incorporation of residues Cys-25 through Tyr-13, and a triple coupling protocol, for amino acids Met-12 through Cys-1. Symmetrical anhydrides were used in crystalline form as described in Yamashiro (1987). Crystalline symmetrical anhydrides (1.0 mmole) were each dissolved in 6 ml DCM and stored in the amino acid reservoirs at 4° C. Sidechain protecting groups used were: Cys, 4-MeBenzyl; Lys, 2-ClZ; Ser, Benzyl; Arg, Tosyl; Thr, Benzyl; Asp, Benzyl; Tyr, 2-Br-Benzyl.

Unless specified, volumes were 8 ml, except for step 2 below, which was 10 ml, and all reactions were carried out at room temperature. After incorporation of the Asp-14 residue, the volume of step 2 was increased to 15 ml while all other volumes were raised to 10 ml after incorporation of the Arg-10 residue. The double coupling protocol consisted of steps 1–16 listed in the Table below.

Amino acids Met-12 through Cys-1 were added by a triple coupling protocol which included, in addition to steps 1–16, steps 17–20 in the MVIIA protocol Table.

MVIIA PROTOCOL TABLE

Step	Reagent
1	DCM wash (3 times)
2	67% TFA/M (20 min.)
3	DCM wash (2 times)
4	25% dioxane/DCM wash (2 times)
5	5% DIEA/DCM wash
6	DCM wash
7	5% DIEA/DCM wash
8	DCM wash (5 times)
9	1.0 mmol SA in DCM (5 min)
10	0.5 mmol IPM in 3 ml TFE plus 1 ml DCM
11	(5 min)
12	0.5 mmol IPM in 5 ml DCM (5 min)
13	DMF wash (3 times)
14	1.0 mmol SA in DMF (5 min)
15	0.5 mmol IPM in 5 ml DCM (5 min)
16	0.5 mmol IPM in 4 ml DMF (5 min)
17	DCM wash
18	DCM wash (2 timnes)
19	1.0 mmol SA in DCM (5 min) 0.5 mmol IPM in 4 ml DMF (5 min)
20	DCM Wash

Crystalline symmetrical anhydrides (1 mmole) were each dissolved in 6 ml DCM or DMF and stored in the amino acid reservoirs at 4° C. Side-chain protecting groups used were: Cys, 4-MeBzl; Lys; ClZ; Ser, Bzl; Arg, tosyl; Thr, Bzl; Asp, Bzl; Tyr, BrZ.

For BOC-Arg(tosyl)-OH, the following mixture was prepared: 1.87 BOC-Arg(tosyl)-OH, 0.57 g 1-hydroxybenzotriazole, 15 ml DMF, stirred to dissolve, cooled to 4° C. added 0.52 ml diisopropylcarbodiimide, and split in half for steps 9 and 13. For this coupling, the protocol was modified as follows: step 8 was 3 times DCM wash and 2 times DMF wash; step 9 was for 10 min; step 11 was for 10 min; step 13 was for 10 min; step 14 was 0.4 mmol IPM in 4 ml DMF for 10 min; step 15 was for 10 min; step 16 was 1 times DMF wash and 1 time DCM wash. Reaction mixtures in steps 9, 10, 13, 14 and 18 were not drained.

The mixture for a third coupling for incorporating the Arg-10 residue consisted of 1.00 g BOC-Arg(tosyl)-OH, 1 ml DMF, 5 ml DCM, stirred to dissolve, and cooled to 4° C. to which is then added 1.67 ml 0.6M DCCI in DCM.

After the last amino acid had been incorporated, the protected peptide resin was subjected to steps 1–4 to remove the N-terminal BOC group, collected on a filter with use of ethanol, and dried in vacuum to yield 2.61 g.

MVIIA has also been successfully synthesized on an ABI 430A synthesizer using slight modifications of the above protocol.

C. Deblocking and Cleavage in Liguid HF

A mixture of protected peptide resin (1.32 g), 2-mercaptopyridine (0.50 g), p-cresol (2.6 g), and liquid hydrogen fluoride (HF) (25 ml) was stirred at 0° C. for 80 min. The liquid HF was evaporated with a rapid stream of nitrogen gas, first below 0° C. then at 24° C. The mixture was stirred in ethyl acetate (25 ml) until a finely divided solid was obtained. The solid was filtered, washed with ethyl acetate, and air dried to yield 1.09 g. This solid was stirred in 50% aqueous acetic acid (10 ml) to dissolve the peptide material, filtered, and washed with 20 ml water. The filtrate was freeze-dried to yield 450 mg of fluffy powder.

D. Formation of Disulfide Bridges

A sample (300 mg) of the fluffy powder was dissolved in 30 ml of 0.05M ammonium bicarbonate, 10 mM dithiothreitol (DTT), and 2M guanidine hydrochloride. The solution, which had a pH of 6.7, was allowed to stand at 24° C. for

2 hr. then diluted with 120 ml of water and stirred for 20 hr at 24° C. DTT (25 mg) was added and the solution allowed to stand at 24° C. for 80 min. The mixture was then stirred at 4° C. for 3 days.

E. Isolation of MVIIA OCT

The solution from Part D was acidified with glacial acetic acid (2 ml), evaporated in vacuo to a low volume, and fractionated by gel filtration on Sephadex G-25 in a 2.5×48 cm column, using 1N acetic acid, to remove peptide polymeric species (exclusion volume), and salts (slowest moving peak). Fractions (5 ml) were collected, with peptide absorbance monitored at 280 nm. Fractions corresponding to the monomer peptide were pooled and freeze-dried to give 127 mg of fluffy powder. A sample of the monomeric material (34 mg) was purified by preparative HPLC on a Vydac 218 TP1022 column with a gradient of 10–20% acetonitrile in 0.1% trifluoroacetic acid over 50 min at 8 ml/min, with detection at 226 nm and collection of 4 ml fractions. Fractions corresponding to the major peak were pooled, evaporated in vacuo to remove acetonitrile, and freeze-dried to yield 7.7 mg. Analytical HPLC on a Vydac 218 TP104 column with the same solvent and gradient over 10 min followed by 10 min of isocratic elution at the 20% composition (1.5 ml/min) gave a single peak identical in behavior to an authentic sample of OCT MVIIA. Amino acid analysis of a 24-hr HCl-hydrolysate gave: Asp, 0.93; Thr,1.05; Ser, 2.85; half-cystine, 5.2; Gly, 4.08; Ala, 1.07; Met 0.94; Leu, 1.02; Tyr,0.85; Lys, 3.98; Arg, 2.09.

F. Radio-Iodination of MVIIA MVIIA peptide was iodinated by reaction with Iodogene™ in the presence of NaI according to Cruz et al., with minor modification. 2 m Ci of carrier-free $Na^{125}I$, 75 ul 0.5M phosphate buffer pH 7.4 and 20 ul of 1 ug/ul peptide were added to a polypropylene test tube coated with 10 ug Iodogenf™. The tube was agitated for 8 minutes, and the solution was chromatographed by HPLC through a 10×0.46 cm C-8 reverse phase column with a pore size of 300 Å (Brownlee Labs, Santa Clara, Calif.). The sample material was eluted with a gradient from 0.1% trifluoroacetic acid to 60% acetonitrile in 0.1% trifluoroacetic acid. The major peak of active radio-iodinated peptide was resolved at about 2 minutes greater retention time than the underivatized peptide.

The fractions containing this peak were collected and later diluted for use in binding experiments. MVIIA, iodinated under the conditions as above except with non-radioactive NaI, was tested for the ability to inhibit depolarization-dependent ATP release from synaptosomes as described in Ahmad and Miljanich (1988) and found to be as potent in this regard as the underivatized peptide.

G. Synthesis of Other OCT Peptides

Synthesis of other OCT peptides was according to the solid-phase method described above, except that a single coupling protocol involving steps 1–12 in Part C was used for coupling the first 10 C-terminal amino acids residues, and a double coupling method involving steps 1–16, Part C was used for coupling the final n-10 N-terminal residues, where n is 24–29. Releasing the peptide from the solid support, removing the blocking groups, and joining the disulfide bridges were carried out substantially as above, or as described in Part H, below. The peptide was separated from salts and polymeric peptide species by gel filtration on Sephadex G-25, and purified on preparative HPLC. For binding studies, each peptide can be radioiodinated essentially as above.

H. Alternate Oxidation Methods

Two alternative oxidation methods were used in the preparation of MVIIA/SNX-111.

1. The lyophilized crude linear peptide was dissolved in 3M guanidine hydrochloride and 1.2M ammonium acetate solution to yield a concentration of approximately 12 mg peptide/ml. DTT was added to a ratio of 15 mg DTT per 100 mg peptide, and the mixture was stirred at room temperature for 1 hour. The solution was diluted 6-fold with distilled water, and stirred at 4° C. for 3–5 days. The progress of peptide oxidation was monitored by HPLC. The endpoint of the oxidation process was the complete disappearance of free thiols, determined by Ellman reaction.

2. The lyophilized crude linear peptide was dissolved in 3M guanidine hydrochloride and 0.3M potassium phosphate solution to yield a concentration of approximately 12 mg peptide/ml. After addition of 40 mg cysteine and 15 mg DTT per 100 mg peptide, the pH of the solution was adjusted to 8.0–8.1 with potassium hydroxide solution. The mixture was stirred at room temperature for 1 hour. The peptide solution was diluted 6-fold with water, and stirred at 40° C. for 3–5 days. The progress of peptide oxidation was monitored by HPLC. The endpoint of the oxidation process was the complete disappearance of free thiols, determined by Ellman reaction. (Method 2 was used in the preparation of SNX-236 and SNX-239).

Following oxidation by either of the above methods, the solution was acidified with acetic acid to pH 3, and lyophilized.

EXAMPLE 2

Omega-conopeptide Binding to Omega-conopeptide Binding Sites in Synaptosomal Membranes

A. Preparation of Mammalian-Brain Synaptosomes and Synaptosomal Membranes

Synaptosomes were prepared from rat whole brain or hippocampal region of brain. Rats were sacrificed, and forebrains were removed and transferred to 10 ml ice-cold 0.32M sucrose containing the following protease inhibitors (PI): 1 mM EGTA; 1 mM EDTA; 1 uM pepstatin; 2 uM leupeptin. Brains were homogenized using a motor-driven Teflon-glass homogenizer (approx. 8 passes at 400 rpm). Homogenates from 4 brains were pooled and centrifuged at 900×g for 10 minutes at 4° C. Supernatants were then centrifuged at 8,500×g for 15 minutes. Resulting pellets were resuspended in 10 ml each ice-cold 0.32M sucrose plus PI with vortex mixing. The suspension was then centrifuged at 8,500×g for 15 minutes. Pellets were resuspended in 20 ml ice-cold 0.32M sucrose plus PI. The suspension (5 ml/tube) was layered over a 4-step sucrose density gradient (7 ml each: 1.2M sucrose, 1.0M sucrose, 0.8M sucrose, 0.6M sucrose; all sucrose solutions containing PI). Gradient tubes were centrifuged in a swinging bucket rotor at 160,000×g for 60 minutes at 4° C. The 1.0M sucrose layer plus the interface between the 1.0 and 1.2M sucrose layers were collected and diluted with ice cold deionized water plus PI to yield a final sucrose concentration of 0.32M. The resulting suspension was centrifuged at 20,000×g for 15 minutes. Pellets were then resuspended in 5 ml icecold phosphate buffered saline plus PI. The resulting rat brain synaptosomes were then aliquoted and stored in a liquid nitrogen containment system.

Prior to use in binding assays, synaptosomes were thawed and diluted with 3 volumes of ice cold deionized water plus PI. This suspension was homogenized using a PT 10-35 Polytron (setting 6) for two 10-second bursts. The homogenate was centrifuged at 40,000×g for 20 minutes at 4° C. The resulting pellets were resuspended in about 5 ml of ice cold phosphate buffered saline plus PI. The resulting brain synaptosomal membrane preparation was aliquoted and stored

at −80° C. until use. Protein concentration of the membrane preparation was determined using Bradford reagent (BioRad), with bovine serum albumin as standard.

B. Saturation Binding Assay MVIIA OCT was radiolabeled with ^{125}I-iodine by reaction with Iodogen™, essentially according to the method of Ahmad and Miljanich (1988). Following the Iodogen reaction, the peptide solution was chromatographed by HPLC through a C-8 reversed phase column and eluted with a gradient from 0.1% trifluoroacetic acid in water to 0.1% trifluoroacetic acid in water/acetonitrile (40:60 vol/vol). The major peak of radioactivity following the underivatized MVIIA OCT was collected.

The binding constant (K_d) for [^{125}I]-MVIIA OCT to rat brain synaptosomal membranes was determined by a saturation binding method in which increasing quantities of [^{125}I] MVIIA OCT were added to aliquots of a synaptosomal membrane preparation (10 ug membrane protein, suspended in binding buffer consisting of 20 mM HEPES, pH 7.0, 75 mM NaCl, 0.1 mM EGTA, 0.1 mM EDTA, 2 μM leupeptin, 0.035 μg/ml aprotinin, and 0.1% bovine serum albumin (BSA), in a total volume of 0.5 ml). Binding at each concentration of labeled compound was determined in the absence and presence of 1 nM unlabeled MVIIA OCT to determine specific binding (as described in part B, below). The amount of labeled peptide specifically bound at each concentration was used to determine B_{max}, the concentration of specific binding sites on the synaptosomes, and K_d, following standard binding analysis methods (Bennett, et al., 1983). Scatchard analysis of saturation binding curve of [^{125}I]MVIIA revealed a K_d of about 10 pM for the compound.

B. Competitive Displacement Binding Assay

1. Competitive Displacement of OCT MVIIA.

Rat brain synaptosomal membranes prepared as described in Part A were suspended in a binding buffer consisting of 20 mM HEPES, pH 7.0, 75 mM NaCl, 0.1 mM EGTA, 0.1 mM EDTA, 2 μM leupeptin, 0.035 μg/ml aprotinin, and 0.1% bovine serum albumin (BSA). [^{125}I]-MVIIA (SNX-111) OCT (25–30,000 cpm, approximately 1500–2000 Ci/mmol) and test compound were aliquoted into polypropylene tubes, in the absence or presence of 1 nM MVIIA (SNX-111) OCT to determine non-specific binding. The membrane suspension was diluted and aliquoted last into the test tubes, such that each assay tube contained 10 μg membrane protein and the total volume was 0.5 ml. After incubation for 1 hour at room temperature, tubes were placed in an ice bath, then filtered through GF/C filters (Whatman), which were presoaked in 0.6% polyethyleneimine and prewashed with wash buffer (20 mM HEPES, pH 7.0, 125 mM NaCl, 0.1% BSA) using a Millipore filtration system. Just prior to filtration, each assay tube received 3 ml ice-cold wash buffer. The filtered membranes were washed with two 3 ml volumes of ice-cold wash buffer, dried, and filter-bound radioactivity was measured in a Beckman gamma counter (75% counting efficiency).

Representative displacement binding curves for rat brain synaptosomal membranes are illustrated in FIG. 3. IC_{50} values were computed from line fit curves generated by a 4-parameter logistic function. These values represent the concentration of test compound required to inhibit by 50% the total specific binding of [^{125}I]-MVIIA (SNX-111) OCT to rat brain synaptosomal membranes, where specific binding is defined as the difference between binding of [^{125}I]-MVIIA (SNX-111) OCT in the absence and presence of excess (1 nM) unlabelled MVIIA OCT. Non-specific binding is that binding of radiolabeled compound which is measured in the presence of excess unlabeled MVIIA OCT. Such values serve as approximations of the relative affinities of a series of compounds for a specific binding site.

2. Competitive Displacement of OCT SVIB.

Rat brain synaptosomal membranes were prepared as described above. OCT SVIB was radiolabeled by iodination with ^{125}I-iodine by the Iodogen reaction. Displacement binding of radiolabeled SVIB on rat brain synaptosomal membranes was carried out as in Example 4B. SVIB displacement curves for several of the omega-conopeptides assayed is shown in FIG. 4. IC_{50} values and relative potency values were calculated as described below. Tables 2 and 3 show the relative potency values for omega-conopeptides examined, and the ratio of relative potencies of the compounds for the OCT MVIIA site and to the SVIB binding site.

The binding constant (K_i) for each test substance was calculated using non-linear, least-squares regression analysis (Bennett and Xie, 1988) of competitive binding data from 2 assays performed in duplicate on separate occasions. The relationship between K_i and IC_{50} (concentration at which 50% of labeled compound is displaced by test compound is expressed by the Cheng-Prusoff equation:

$$K_i = IC_{50}/(1+[L]/K_d)$$

where IC_{50} is the concentration of test substance required to reduce specific binding of labeled ligand by 50% ; [L] is the concentration of [^{125}I]-MVIIA (SNX-111) OCT used in the experiment; and K_d is the binding constant determined for binding of [^{125}I]-MVIIA (SNX-111) OCT to rat brain synaptosomal membranes in saturation binding experiments. Table 3 summarizes computed IC_{50} for various omega-conopeptides for the MVIIA binding site of rat brain synaptosomal membranes.

Relative potency for displacement of binding is calculated as a ratio of the IC_{50} of the test compound and the IC_{50} of the reference compound. The reference compound is generally the unlabeled equivalent of the labeled ligand. Calculation of relative potency is as follows:

$$[\log(\text{relative potency})] = \log(IC_{50(ref)}) - \log(IC_{50(test)})$$

Relative potency values for binding at OCT MVIIA (SNX-111) and OCT SVIB (SNX-183) sites are listed in Table 3.

EXAMPLE 3

Inhibition of Electrically Stimulated Contractions of Guinea Pig Ileum

Guinea pigs (300–400 gms) were decapitated and the ileum removed. A section of ileum about 6 cm from the caecum was placed immediately into Krebb's modified buffer maintained at 37° C. in a water bath, and aerated with a mixture of 95% O_2 and 5% CO_2. The buffer contains: KCl, 4.6 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; Glucose, 10.0 mM; NaCl 118.2 mM; $NaHCO_3$, 24.8 mM; $CaCl_2$, 2.5 mM.

Small pieces of ileum were cut and pulled over a glass pipette, scored and the longitudinal muscle removed. Each

piece was attached to an electrode at one end and to a force transducer at the other end. The preparation was lowered into an organ bath maintained at 37° C. and aerated with O_2:CO_2. The resting tension was set at 1 gm. and the tissue was stimulated at 30–50 V with a duration of 4.5 msec per stimulation.

Baseline responses (contractions) were recorded for 10–15 min. and aliquots (100 ml) of drug were added to the bath until inhibition occurred. Following testing, tissues were washed until original response magnitude was achieved.

EXAMPLE 4

Rat Model of Peripheral Neurovathy

Male Sprague-Dawley rats (250–350 gm) were prepared with chronic lumbar intrathecal catheters inserted under halothane anesthesia (Yaksh and Rudy, 1976). Animals were placed in a prone position and the left paraspinal muscles were separated from the spinous processes at the L_4-S_2 levels, as described by Kim and Chung (1992). The left L5and L6nerve roots were exposed and tightly ligated with 6–0 surgical silk suture. Approximately 7–10 days after ligaition, the lumbar subarachnoid space was catheterized with saline-filled polyethylene (PE-10) tubing as described by Yaksh and Rudy (1976). The Catheter was anchored with stay sutures to the adjacent muscle tissue where it emerged from the cisterna magna. Animals were given at least 3 days to recover before assessing mechanical allodynia thresholds. Allodynia was typically observed to occur beginning 1–2 days post-surgery and continued for as long as 45 days. Animals showing motor deficits were excluded from further study.

For testing, animals were placed in plastic cubicles with open wire mesh bottoms. Compound dissolved in preservative-free saline solution was administered in a volume of 10 μl through the intrathecal catheter, followed by 10 μl saline to flush the catheter line. Animals were tested for allodynia at various time points after drug treatment, as described below.

To assess the threshold of a non-noxious stimulus required to produce a left hind paw withdrawal (allodynia), Von Frey hairs (ranging from 0.4–15 grams), were systematically applied to the surgically treated plantar of the hind paw. The hair was held against the surface with sufficient force to cause slight bending and held for 6–8 seconds. Failure to evoke a response was cause to test the next stiffer hair. Evocation of a brisk withdrawal response was cause to test the next lower stimulus intensity. This paradigm was repeated according to a statistical method (Dixon, 1976) to define the 50% response threshold. Allodynia was evidenced by a threshold less than 3 grams (referring to the hair stimulus intensity) exhibited by all surgically treated animals.

Results of animals treated with saline, or various doses of omega-conopeptides are shown in FIG. 6, FIG. 7 and FIG. 8. Data in FIG. 6 are expressed as percent maximum effect, where the maximum effect indicates a complete reversal of allodynia, or insensitivity to stimulus (maximum equals 15 gram hair cutoff). A baseline of zero indicates a mean sensitivity less than 3 grams. As shown in FIG. 6, treatment of rats (n=6/treatment) with 1 or 3 μg SNX-111 resulted in elevation of threshold response. Peak effects were observed by 30–60 minutes, and effects lasted in excess of 60 minutes.

FIGS. 8A and 8B show results of tests in which animals were given a single intrathecal bolus injection of SNX-273 (0.1 μg, 0.3 μg, or 1 μg), SNX-279 (0.1 μg, 0.3 μg, or 1 μg), SNX-111 (0.1 μg) or vehicle (0.9% Sodium Chloride injection, USP, Sanofi Animal Health, Inc., Overland Park, Kans.). Test control compounds were delivered in a volume of 10 μl followed by 10 μl of saline to flush the catheter. Results of allodynia tests, performed as described above, are shown as percentage of maximum possible effect (MPE):

$$\%MPE = \frac{\text{New Threshold(g)} - \text{Baseline Threshold(g)}}{15 \text{ grams} - \text{Baseline Threshold}} \times 100$$

According to this analysis, the higher the %MPE, the better the antinociceptive effect. As shown in FIGS. 8A and 8B, SNX-111, SNX-273 and SNX-279 each blocked mechanical allodynia significantly in comparison to saline control (asterisks in the figures indicate statistically significant differences between treatment and saline, $p<0.05$, Student's t test). The apparent order of potency for suppression of allodynia is SNX-111=SNX-273>SNX-279. This is consistent with the compounds' relative affinities at the SNX-111 binding site (IC_{50}'s: SNX-111, 8 pM; SNX-273, 8 pM; SNX-279, 40 pM).

Animals were also observed for the appearance of general motor dysfunction, as evidenced by inability to ambulate symmetrically and for any other overt signs of unusual activity. No effects on motor activity were observed in saline-treated animals; a dose-dependent tremor characteristic of SNX-111 administration was observed in animals given SNX-111.

EXAMPLE 5

Methionine-Lactate Buffer Formulations

Analgesic efficacy of spinally-administered SNX-111 was tested using a methionine-lactate buffer formulation in the paradigm detailed in Example 4. SNX-111 (10 μg/ml) and L-methionine (50 μg/ml) were dissolved in a vehicle comprised of sodium lactate (150 mM) adjusted to pH 4–4.5 with 250 mM lactic acid. This formulation was used to deliver 0.1 μg SNX-111 intrathecally, as described in Example 5 at 30, 60, 120 and 240 minutes after treatment with test or control compound. FIG. 9 shows effects on mechanical allodynia of a single intrathecal bolus injection of 10 μl saline (open circles) or lactate buffer (150 mM) containing 50 μg/ml methionine with (closed squares) or without (closed triangles) 10 μg/ml SNX-111. Neither saline alone or methionine lactate control buffer alone was effective to suppress allodynia, whereas the SNX-111 formulation was effective in this regard (FIG. 9). Moreover, it was observed that %MPE values for saline-treated controls were not significantly different from those of animals given methionine-lactate buffer alone.

Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled that various changes and modifications can be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 36

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: MVIIA/SNX- 111, FIGURE 1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Lys Gly Lys Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys
1 5 10 15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: MVIIB/SNX- 159, FIGURE 1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Lys Gly Lys Gly Ala Ser Cys His Arg Thr Ser Tyr Asp Cys Cys
1 5 10 15

Thr Gly Ser Cys Asn Arg Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: GVIA/SNX- 124, FIGURE 1

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site

-continued

(B) LOCATION: 21
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Lys Ser Xaa Gly Ser Ser Cys Ser Xaa Thr Ser Tyr Asn Cys Cys
1 5 10 15

Arg Ser Cys Asn Xaa Tyr Thr Lys Arg Cys Tyr
20 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: GVIIA/SNX- 178, FIGURE 1

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Lys Ser Xaa Gly Thr Xaa Cys Ser Arg Gly Met Arg Asp Cys Cys
1 5 10 15

Thr Ser Cys Leu Leu Tyr Ser Asn Lys Cys Arg Arg Tyr
20 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: RVIIA/SNX- 182, FIGURE 1

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys Lys Pro Xaa Gly Ser Xaa Cys Arg Val Ser Ser Tyr Asn Cys Cys
1 5 10 15

Ser Ser Cys Lys Ser Tyr Asn Lys Lys Cys Gly
20 25

(2) INFORMATION FOR SEQ ID NO:6:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SVIA/SNX- 157, FIGURE 1

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Arg Ser Ser Gly Ser Xaa Cys Gly Val Thr Ser Ile Cys Cys Gly
1 5 10 15

Arg Cys Tyr Arg Gly Lys Cys Thr
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: TVIA/SNX- 185, FIGURE 1

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Leu Ser Xaa Gly Ser Ser Cys Ser Xaa Thr Ser Tyr Asn Cys Cys
1 5 10 15

Arg Ser Cys Asn Xaa Tyr Ser Arg Lys Cys Arg
20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SVIB/SNX- 183, FIGURE 1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

-continued

```
Cys Lys Leu Lys Gly Gln Ser Cys Arg Lys Thr Ser Tyr Asp Cys Cys
1               5                  10                  15

Ser Gly Ser Cys Gly Arg Ser Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-190, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Cys Lys Gly Ala Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-191, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Cys Ala Gly Ala Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-193, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Cys Lys Gly Ala Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys Gly
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids

-continued

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-194, FIGURE 2

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= "where X is Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Cys Lys Gly Ala Gly Ala Lys Cys Ser Arg Leu Xaa Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-195, FIGURE 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Cys Lys Gly Ala Gly Ala Lys Cys Ser Arg Leu Xaa Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Ala Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-196, FIGURE 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Asn Cys Lys Gly Ala Gly Ala Lys Cys Ser Arg Leu Xaa Tyr Asp Cys
1               5                  10                  15

Cys Thr Gly Ser Cys Arg Ser Gly Ala Cys Gly
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

-continued

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-197, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Asn Ser Cys Lys Gly Ala Gly Ala Lys Cys Ser Arg Leu Xaa Tyr Asp
1               5                  10                  15

Cys Cys Thr Gly Ser Cys Arg Ser Gly Ala Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-198, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Cys Lys Gly Lys Gly Ala Lys Cys Ser Arg Leu Met Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Ala Ser Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-200, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Cys Lys Gly Ala Gly Ala Ala Cys Ser Arg Leu Met Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-201, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Cys Lys Gly Lys Gly Ala Lys Cys Arg Lys Thr Ser Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
            20                  25
```

-continued

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-202, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Cys Lys Leu Lys Gly Gln Ser Cys Ser Arg Leu Met Tyr Asp Cys Cys
1 5 10 15

Ser Gly Ser Cys Gly Arg Ser Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-207, FIGURE 2

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Cys Leu Ser Xaa Gly Ser Ser Cys Ser Arg Leu Met Tyr Asn Cys Cys
1 5 10 15

Arg Ser Cys Asn Xaa Tyr Ser Arg Lys Cys Arg
20 25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-231, FIGURE 2

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Lys Gly Lys Gly Ala Xaa Cys Arg Lys Thr Met Tyr Asp Cys Cys

-continued

```
1               5                  10                  15

Ser Gly Ser Cys Gly Arg Arg Gly Lys Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP I FRAGMENT

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Cys Lys Gly Lys Gly Ala
1               5
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP I FRAGMENT

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Cys

1
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP I FRAGMENT

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Tyr Asp Cys Cys Thr Gly Ser Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP I FRAGMENT

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Arg
1

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP 1 FRAGMENT

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Lys Cys
1

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP 2 FRAGMENT

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "where X is hydroxyproline"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Leu Ser Xaa Gly Ser Ser Cys Ser
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP 2 FRAGMENT

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Tyr Asn Cys Cys Arg Ser Cys Asn
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-230, FIGURE 1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Lys Gly Lys Gly Ala Pro Cys Arg Lys Thr Met Tyr Asp Cys Cys
1 5 10 15

Ser Gly Ser Cys Gly Arg Arg Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-236, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Leu Ser Xaa Gly Ser Ser Cys Ser Arg Leu Met Tyr Asn Cys Cys
1 5 10 15

Arg Ser Cys Asn Pro Tyr Ser Arg Lys Cys Arg
20 25

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: CONOPEPTIDE GROUP 2 FRAGMENT

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Ser Arg Lys Cys Arg
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-239, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Cys Lys Gly Lys Gly Ala Lys Cys Ser Leu Leu Met Tyr Asp Cys Cys
1 5 10 15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
20 25

-continued

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-199, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Lys Gly Lys Gly Ala Lys Cys Ser Ala Leu Met Tyr Asp Cys Cys
1 5 10 15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX 240, FIGURE 2

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "The cysteine residue carries an acetyl group"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Cys Lys Gly Lys Gly Ala Lys Cys Ser Leu Leu Met Tyr Asp Cys Cys
1 5 10 15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(v i) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-273, FIGURE 2

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Cys Lys Gly Lys Gly Ala Lys Cys Ser Arg Leu Ala Tyr Asp Cys Cys
1 5 10 15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
20 25

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: SNX-279, FIGURE 2

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= "where X is sulfoxy-methionine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Cys Lys Gly Lys Gly Ala Lys Cys Ser Arg Leu Xaa Tyr Asp Cys Cys
1               5                  10                  15

Thr Gly Ser Cys Arg Ser Gly Lys Cys
            20                  25
```

It is claimed:

1. A stable omega conopeptide formulation comprising an omega conopeptide and an anti-oxidant composition capable of preventing methionine oxidation.

2. The formulation of claim 1, wherein the anti-oxidant composition includes a carboxylic acid buffer.

3. The formulation of claim 2, wherein the carboxylic acid buffer is lactate buffer.

4. The formulation of claim 1, wherein the anti-oxidant is methionine.

5. The formulation of claim 1, wherein the anti-oxidant composition includes lactate buffer and methionine.

6. An omega-conopeptide SNX-273 having the sequence: SEQ ID NO: 35.

7. An omega conopeptide SNX-279 having the sequence SEQ ID NO: 36.

* * * * *

UNITED STATES DEPARTMENT OF COMMERCE
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Attachment E

Customer Num: 204

COMPUTER PACKAGES, INC.
414 HUNGERFORD DRIVE
ROCKVILLE MD 20850

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "STAT", below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "STAT" below. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

PATENT NUMBER	FEE CODE	FEE AMT	SUR CHARGE	APPLICATION NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT	ATTY DKT NUM
5,795,864	183	$880.00	$0.00	08/496,847	08/18/98	06/27/95	04	NO	PAID	5865-0009.31

DIRECT YOUR RESPONSE TOGETHER WITH ANY QUESTIONS ABOUTTHIS NOTICE TO:
Mail Stop: M. Correspondence, Director of the United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

BRIEF DESCRIPTION OF ACTIVITIES DURING REGULATORY REVIEW PERIOD FOR PRIALT®

Date	Action
June 30, 1994	Submission of IND 45718
July 12, 1994	FDA Acknowledgment of receipt on July 5, 1994
August 4, 1994	IND effective date
August 5, 1994	FDA request for information
November 21, 1994	Protocol amendment in response to FDA request of August 5, 1994
December 14, 1994	Received FDA comments on Protocol Amendment
January 30, 1995	Protocol amendment adding new investigator
February 10, 1995	Protocol amendment adding new investigator
March 14, 1995	Submission of Safety Report
April 7, 1995	Correspondence to FDA requesting clarification on patient population criteria
May 11, 1995	Teleconference with FDA
May 19, 1995	Protocol revision in response to May 11, 1995 teleconference
June 15, 1995	Submission of Safety Report
August 16, 1995	Submission of Annual Report
September 27, 1995	Request meeting with FDA
November 1, 1995	Meeting with FDA
November 7, 1995	Submission of additional information to FDA following meeting of November 1, 1995
November 17, 1995	Submission of November 1, 1995 meeting minutes
November 30, 1995	Phone call with FDA discussing protocol design
December 21, 1995	Protocol amendment adding new investigator
January 18, 1996	Protocol revision and information submitted
February 1, 1996	Letter from FDA allowing study to proceed
February 14, 1996	Request for meeting to discuss pre-clinical program
March 8, 1996	Submission of non-clinical background
April 11, 1996	Protocol amendment adding new investigator
May 1, 1996	Phone call with FDA
May 23, 1996	Phone call with FDA
June 24, 1996	Protocol amendment adding new investigator
July 10, 1996	New protocol submission
July 23, 1996	New protocol submission
August 12, 1996	Letter from CDER referencing July 23, 1996 letter
September 12, 1996	Letter to FDA in response to August 12, 1996 letter
October 9, 1996	Information amendment – chemistry and microbiology
October 28, 1996	Annual Report submitted
November 8, 1996	Protocol amendment adding new investigator
December 5, 1996	Pharmacology/Toxicology information submitted
January 9, 1997	Protocol amendment adding new investigator
January 23, 1997	Phone call with FDA in preparation for March Advisory

Date	Action
	Committee meeting
January 30, 1997	Letter from FDA proposing questions for the Advisory Committee meeting
February 13, 1997	Protocol amendment adding new investigator
February 22, 1997	FDA request for pharmacology information
March 19, 1997	Submission to FDA in response to letter of February 22, 1997
April 25, 1997	Submission of official transcript of Advisory Committee Meeting
May 16, 1997	Protocol amendment adding new investigators
June 30, 1997	Protocol amendment adding new protocol
July 21, 1997	Protocol amendment adding new investigator
August 15, 1997	New protocol submitted: A Multicenter, Phase II, Placebo-Controlled Pilot Study of SNX-111 for epidural administration
September 25, 1997	Protocol amendment adding new investigators
October 3, 1997	Submission of Annual Report covering September 1996 through June 1997
November 18, 1997	Submission of Final Report on Study 94-004-02
December 23, 1997	Phone call from FDA requesting information on protocols
January 13, 1998	Letter to FDA in response to phone call of December 23, 1997
January 22, 1998	Letter to FDA requesting meeting to discuss results of interim analysis for Protocol 96-002
February 5, 1998	Phone call to FDA requesting status of meeting request made on January 22, 1998
February 26, 1998	Protocol amendment adjusting sample size
March 13, 1998	Phone call from FDA indicating that letter is being drafted
April 1, 1998	Letter from FDA referring to submissions of January 22 and February 26, 1998
April 10, 1998	Phone call from FDA requesting additional information on Protocols 96-002 and 95-002
April 13, 1998	Response to FDA request of April 10, 1998 for information
May 22, 1998	Response to FDA request for information with supplemental background material for FDA meeting
June 18, 1998	Phone call from FDA confirming July 23, 1998 meeting
July 23, 1998	Meeting with FDA
August 18, 1998	Submission of meeting minutes from July, 23, 1998
September 28, 1998	Submission of telephone minutes of call on September 23, 1998
November 5, 1998	Protocol amendment adding new investigator
November 18, 1998	Letter from FDA requesting information
December 1, 1998	Letter to FDA in response to November 18, 1998, request for information
December 14, 1998	Phone conference with FDA regarding toxicology studies
January 7, 1999	Letter to FDA informing of name change from Neurex to Elan Pharmaceuticals, Inc.
January 20, 1999	Letter from FDA acknowledging receipt of correspondence and requesting meeting to clarify pharm/tox issues

Date	Action
January 28, 1999	Teleconference with FDA regarding pharm/tox issues
January 29, 1999	Request for teleconference with FDA to follow up with issues raised in January 28, 1999 call
February 3, 1999	Letter from FDA acknowledging request for meeting
February 16, 1999	Teleconference with FDA discussing safety issues and requirements for NDA
March 7, 1999	Submitted response to FDA request for information
March 18, 1999	Letter from FDA with minutes from telephone conference on February 16, 1999, and scheduling meeting for April 8, 1999
April 5, 1999	Letter to FDA regarding pre-NDA meeting topics
April 8, 1999	Pre-NDA meeting with FDA
April 16, 1999	Letter from FDA requesting a teleconference to discuss carcinogenicity
April 22, 1999	Teleconference with FDA per request of April 16, 1999
May 17, 1999	Received copy of minutes from FDA of meeting on April 8, 1999
May 21, 1999	Received copy of minutes from FDA of meeting on April 22, 1999
June 17, 1999	Submission of background document on Chemistry, Manufacturing and Control information
June 22, 1999	FDA letter requesting study data be made available during NDA review
July 14, 1999	Information amendment submitting revised Investigator's Brochure
July 17, 1999	Submitted response to FDA request for information of April 8, 1999 pre-NDA meeting
August 2, 1999	Per FDA request, submission of summary of correspondence received from FDA
September 28, 1999	Submitted final protocol for Syrian Hamster Embryonic (SHE) cell transformation assay
October 8, 1999	Annual Report covering August 1, 1998, through August 27, 1999, submitted
November 16, 1999	Submitted overall table of clinical studies as requested by FDA
December 14, 1999	FDA letter discussing review of SHE cell assay
December 28, 1999	Full submission of NDA 21-060
January 10, 2000	FDA acknowledge receipt of NDA on December 28, 1999
February 7, 2000	Protocol amendment increasing enrollment of study patients
March 3, 2000	Protocol amendment adding new investigator
April 4, 2000	FDA clinical request for information to continue evaluation of NDA
April 6, 2000	Submitted response to FDA request of April 4, 2000
May 16, 2000	Protocol amendment updating investigator information
June 8, 2000	Submitted sample label to FDA
June 27, 2000	Approvable Letter for NDA 21-060 received from FDA
June 30, 2000	Response to FDA Approvable Letter of intent to address

Date	Action
	deficiencies set forth therein
July 25, 2000	Letter from FDA acknowledging receipt of NDA correspondence and meeting request
August 15, 2000	Protocol Amendment with new and updated investigators
September 29, 2000	Response to FDA request for information regarding Approvable Letter of June 27, 2000
October 13, 2000	Submission of safety and efficacy data for NDA
November 7, 2000	Letter from FDA regarding review of submissions dated September 21, 2000 and October 13, 2000
November 29, 2000	Submission of proposed trademark
December 28, 2000	Letter from FDA regarding the Investigator's Brochure
January 26, 2001	Submission of NDA amendment
February 2, 2001	Submission of Annual report covering August 28, 1999 through July 31, 2000
March 28, 2001	Submission of investigator update for Protocols 95-002 and 98-022
April 4, 2001	FDA request for information regarding data
April 26, 2001	Response to April 4, 2001, FDA request for information
May 1, 2001	FDA letter accepting PRIALT as tradename
June 6, 2001	Protocol amendment with investigator update
July 25, 2001	FDA Approvable letter of marketing application
August 3, 2001	Letter to FDA with intent to respond to issues in Approvable Letter of July 25, 2001
September 6, 2001	Letter from FDA requesting information
September 17, 2001	Letter to FDA requesting end of review meeting
October 2, 2001	Letter from FDA acknowledging meeting request
November 20, 2001	Submission of briefing package for December 3, 2001 meeting
December 3, 2001	Meeting with FDA
December 18, 2001	Received minutes from December 3, 2001 meeting
December 20, 2001	Annual Report covering August 1, 2000 through July 31, 2001
January 9, 2002	Letter from FDA setting Chemistry, Manufacturing and Control meeting for February 27, 2002
February 27, 2002	Meeting with FDA
March 8, 2002	Submitted copies of slides presented in February 27, 2002 meeting
April 4, 2002	Submitted new protocol and new investigator
May 10, 2002	Phone call from FDA responding to request for status update
June 25, 2002	Submission of safety report
July 29, 2002	Letter from FDA requesting information
August 21, 2002	Submission of Protocol Amendment
September 4, 2002	Submission of new investigators
October 7, 2002	Letter to FDA in response to request for information of July 29, 2002
November 1, 2002	Protocol Amendment adding new investigator

Date	Action
December 3, 2002	Response to FDA Approvable Letter of July 25, 2001 addressing questions
January 28, 2003	Annual Report covering August 1, 2001 through July 31, 2002
February 20, 2003	Submission of pre-NDA meeting package for meeting on March 25, 2003
March 25, 2003	Meeting with FDA
April 17, 2003	Protocol Amendment
May 2, 2003	Submission of response to pre-NDA meeting of March 25, 2003
June 4, 2003	Letter from FDA commenting on clinical protocol
July 25, 2003	Teleconference with FDA discussing teratology study
August 7, 2003	Letter from FDA scheduling meeting for September 12, 2003
September 12, 2003	Meeting with FDA
October 7, 2003	Letter from FDA with minutes of September 12, 2003 meeting
November 19, 2003	Annual Report for August 1, 2002 through July 31, 2003
December 11, 2003	Protocol Amendment
January 30, 2004	Protocol Amendment
March 26, 2004	Protocol Amendment with investigator update
April 27, 2004	Protocol Amendment with investigator update
May 28, 2004	Protocol Amendment with investigator update
June 25, 2004	Submission of NDA amendment and response to Approvable Letter
July 15, 2004	Protocol Amendment
August 5, 2004	FDA acknowledgement letter of NDA amendment
September 30, 2004	Annual Report covering August 1, 2003 through July 31, 2004
October 27, 2004	FDA request for information
November 1, 2004	Response to FDA request for information of October 27, 2004
December 28, 2004	FDA Approved for commercial marketing

PATENT
Atty. Docket No.: 8576.0068

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 5,795,864)
)
Issued: August 18, 1998)
)
To: Gary Athur Amstutz, Stephen Scott Bowersox,)
Kishorchandra Gohil, Peter Isadore Adriaenssens,)
Ramasharma Kristipati)
)
Assignee: ELAN Pharmaceuticals, Inc.)
)
For: STABLE OMEGA CONOPETIDE FORMULATIONS)

MAIL STOP PATENT EXT.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Attachment G

Sir:

CERTIFICATION

I, CHARLES E. VAN HORN, do hereby certify that this accompanying application for extension of the term of U.S. Patent No. 5,795,864 under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and two (2) copies thereof.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Date: February 22, 2005

By: Charles E Van Horn
Charles E. Van Horn
Reg. No. 40,266